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# ANATOMY OF SUGARCANE STALK AS INFLUENCED BY TOP BORER (*SCIRPOPHAGA NIVELLA* F.) ATTACK

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## I. INTRODUCTION

THE reaction of the tissues of sugarcane stem to mechanical injury sustained as a result of an attack by top borer (*Scirpophaga nivella* F.) and their subsequent structural variations, so far as the authors are aware, have not been reported anywhere. Pemberton (1925) and Stewart and Hansson (1928) reported injury to roots by centipedes and nematodes and there are passing references by Ferret (1927) and Lyon (1927), about the formation of galls in sugarcane stem, the internal structure of which has not been paid the attention it deserves. Morbid anatomy induced by various pathogens and insect pests explains the behaviour of the host to a great extent.

## II. MATERIAL AND METHODS

Four to five shoots attacked by top borer of Co 205, Co 210, Co 213, Co 299, Co 313 and Co 331 were collected before the secondary invasion by other organisms took place. The affected portion of the stalk was cut into internodes at various stages of growth. Hand sections were stained with Safranin and mounted in Canada balsam, in the usual way.

## III. OBSERVATIONS

The examination of slides showed that the reaction of sugarcane tissues to the attack of top borer was confined to the neighbourhood of the actual damage, and the maturer the tissue, the less the area involved; and so also, the less the deviation from normal structure. Both the parenchymatous matrix and vascular bundles were more or less equally affected. The derangement of the tissues manifested itself mainly by the changes in size and shape of cells and the staining quality of their walls.

In very young top-most internodes, two or three layers of parenchymatous cells adjacent to the tunnel usually grew into long palisade-like tissue (Plate I, Fig. 1) deep into the cavity which became irregular in outline, some times their distal ends forming knobs. The outgrowths brought about by the rapid increase in the size of newly formed cells, in the absence of proper tissue tension all round them, narrowed the borer tunnel to a mere slit in some internodes. Safranin stained the walls of the morbid

cells much more deeply than those of the healthy ones. In partially mature internodes, where the growth rate had slowed down, these cells could not acquire their normal size though there was not much difference between a healthy and an affected cell as regards their shape (Plate I, Fig. 2). In very young internodes also this type of reaction by parenchymatous tissue situated between the palisade-like and healthy tissue was sometimes met with. The staining quality of the cell wall also changed as stated above. In fully mature internodes, the reaction to the attack and the presence of a larva was confined to about half a dozen layers of cells all round the cavity. The cell walls became thick and highly lignified (Plate I, Fig. 3) with the result that the healthy tissue was cut off from the cavity and its contents. A prototype of this lignified ring between the healthy tissue and the morbid growth was often met with in younger internodes also. Sometimes the cells outside the sanitary cordon, lost their rounded shape and became polygonal (Plate I, Fig. 4) thus obliterating the intercellular spaces, which appeared to be a further attempt at the segregation of healthy tissues. Excepting the radial elongation of a few cells isolated or in groups, there was no change in the shape or size of the parenchymatous cells surrounding the borer tunnel in fully developed internodes (Plate I, Fig. 4).

Disruption in the case of vascular bundles was equally extensive. There were no vascular bundles in the deranged parenchymatous matrix, due to the complete disorganisation of the primordia of vascular bundles (Plate I, Figs. 1 and 2). Their frequency and structure slowly moved towards the normal as one receded from the cavity. Elongation of xylem end of the vascular sheaths towards the tunnel, the suppression of protoxylem vessels, and distortion of one or both of the metaxylem vessels or their complete absence were among the most common deformities (Plate I, Fig. 5). Phloem was not so easily affected as xylem. Sometimes its place was found to have been occupied by thick-walled cells probably intruders from the adjoining vascular sheath. The sclerenchymatous cells forming the sheath which were so characteristically polygonal in outline when healthy, became diamond-shaped in cross-section with their longer axis towards the cavity. Their walls could not acquire the usual thickness and were comparatively poorly lignified. Vascular bundles far removed from the centre of disturbance in the growing internodes were found to be quite normal in all respects (Plate I, Fig. 5). But in mature internodes even those abutting the cavity did not show any structural change (Plate I, Figs. 3 and 4). Very often their xylem components, less so the phloem, were found to have been choked with a dark, darkish brown or chocolate coloured substance which was insoluble in water, various grades of alcohol and xylol. Sometimes this

substance filled a few of the parenchymatous cells adjacent to the cavity. But in the case of the vascular bundles, it was found to be present at considerable distance from the cavity (Plate I, Fig. 6).

No marked difference in the behaviour of tissues of the varieties studied, was noticeable except that the choking of vascular bundles appeared to be more common in Co 210 than in others.

#### IV. SUMMARY

1. The stage of growth at which the tissues were attacked determined the affected area and the changes in the size and shape of cells forming them.

2. Lignification of cell walls was greater and less respectively in affected parenchyma and sclerenchyma than in the healthy ones.

3. No marked varietal differences in the behaviour of tissues were discernible.

#### V. ACKNOWLEDGEMENTS

The work was carried out as part of the Sugarcane Research Scheme in Bihar being financed jointly by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due.

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#### EXPLANATION OF PLATE

*Microphotographs of Transverse Sections showing Reactions of Stem Tissues at Various Stages of Plant Growth*

(Magnification  $\times 50$  for all)

- FIG. 1. Young internode showing abnormally elongated cells of the parenchymatous matrix without any vascular bundle.
- FIG. 2. Partially mature internode. The tunnel is surrounded by a few layers of parenchymatous cells smaller than the healthy ones. (a) a partially developed vascular bundle with protoxylem suppressed, metaxylem not fully formed, and poorly lignified vascular sheath.
- FIG. 3. Mature internode, showing a highly lignified ring of cells cutting off the tunnel from healthy tissue.
- FIG. 4. Mature internode. The parenchymatous cells become polygonal. A few of those abutting the cavity become elongated; vascular bundles normal.
- FIG. 5. Immature internode showing common deformities of vascular bundles, such as elongation of xylem-end of vascular sheath, suppression of protoxylem and distortion or suppression of metaxylem.
- FIG. 6. Mature internode. Vascular bundles normal but choked with a blackish substance,



# RESPIRATION OF SUGARCANE IN RELATION TO ESSENTIAL PLANT NUTRIENTS

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Received May 28, 1947

## I. INTRODUCTION

MITSCHERLICH and colleagues (quoted from Willcox, 1937) have shown that from the point of view of nutrient requirements, nitrogen element is the one that is required in maximum quantities followed by potash, phosphorus and other elements such as calcium, magnesium, sodium, silicon, iron, etc. Other workers have studied the effect of deficiency of essential elements on the respiratory activity of plant. Thus, Gregory and Richards (1929) and later on Richards (1932) studied the effect of manurial deficiency and observed that the curve of response exhibited a fall soon after the element became deficient. Similarly Lyon (1926, 1927) studied the effect of phosphatic deficiency on plant respiration. Hammer (1936) described the effect of nitrogen supply on the rate of metabolism in plants and showed a marked increase in the respiration rate on the application of nitrates to tomato and wheat plants. Arnon (1937) reported an increased rate of respiration even in the case of excised barley roots on the application of nitrates. Warburg and Negelani (Arnon, 1937) showed that the respiratory quotient of *Chlorella* increased following the absorption of nitrates. Nightingale, *et al.* (1931) observed the effect of calcium deficiency on nitrate absorption and on metabolism in tomato plants. Studies by Parija and Saran (1934) indicated that starved leaves exhibited an enhanced rate of respiration in a deficient environment.

Suzaki and Kenjo (1935, 1936, 1937), Kenjo (1938) and Saito and Kenjo (1939) published the results of their experiments on the effect of deficiency of nitrogen and phosphorus on leaf colour, growth of stalks and leaves, development of roots, sucrose content of juice, total ash content, etc. These experiments were performed in water cultures but the effect of nitrogen and phosphorus deficiency on respiration rate was not determined.

In the studies reported hereunder, an attempt was made to study the metabolic rate of excised shoots of plants raised in sand cultures deficient

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in nitrogen, potash and phosphorus and in the absence and presence of minor elements. Dry weight of plants was determined at the close of the experimental period to elucidate the interrelationship among the type of nutrient medium and respiratory process and growth of plants. And, therefrom, it was proposed to indicate the degree to which the various elements act as limiting factors in plant metabolism.

## II. MATERIAL AND METHODS

The experiments were performed in glass jars measuring 24" × 18" × 10". These were filled with quartz sand that had been washed repeatedly to get rid of all extraneous matter. Equal weighed quantities of sand were put in each of the glass jars, enough of water being added to saturate the sand to its full capacity. Three one-eyed setts obtained from central three nodes of fully developed cane stalks were planted on 3-11-38 at 2½" depth in each of the glass jars. The setts were 2" in length and all weighed approximately equal, the range in the weights being 31.12 and 27.08 grammes. The germination of setts was duly recorded day after day. Most of the setts germinated within a week of the germination of the first sett in the experiment, but another week was allowed before the rest of the ungerminated setts were taken out. The pots were then so arranged (Table I) that the distribution of plants might as far as possible be equal with regard to date of germination.

TABLE I

*Showing the arrangement of pots for various nutrient treatments (Nov., 1938)*

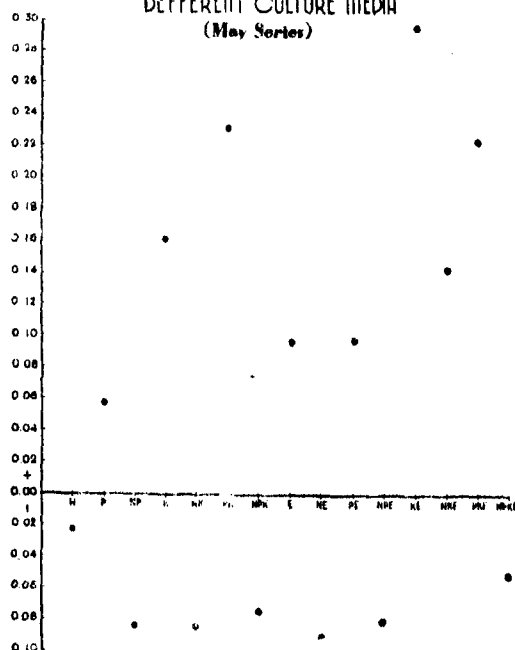
Sl. No.	Treatment	Reference	Pot No.	Date of germination			Pot No.	Date of germination			Pot No.	Date of germination		
				1	2	3		1	2	3		1	2	3
1	Control	.. A	26	18th	16th	16th	21	14th	18th	23rd	28	18th	..	..
2	N	.. B	13	21st	16th	15th	9	19th	20th	16th	35	16th	21st	25th
3	P	.. C	1	14th	13th	12th	2	19th	19th	15th	30	14th	23rd	25th
4	N P	.. D	7	14th	15th	14th	24	14th	..	..	16	23rd	20th	..
5	K	.. E	38	13th	16th	12th	41	25th	18th	17th	19	..	23rd	20th
6	N K	.. F	3	13th	17th	13th	32	19th	19th	19th	17	19th	20th	..
7	P K	.. G	4	14th	16th	14th	40	19th	26th	15th	44	20th	23rd	20th
8	N P K	.. H	10	20th	20th	16th	39	20th	..	..	46	18th	17th	17th
9	E	I	6	21st	19th	14th	14	..	21st	18th	37	14th	..	..
10	N	E J	15	16th	21st	22nd	25	..	..	17th	43	18th	17th	15th
11	P	E K	8	16th	14th	18th	31	..	17th	..	45	..	17th	17th
12	N P	E L	11	21st	21st	17th	42	19th	17th	14th	47	14th	25th	26th
13	K E	M	5	16th	14th	19th	27	15th	21st	19th	29	..	..	26th
14	N K E	N	18	15th	14th	18th	33	18th	19th	20th	48	18th	21st	..
15	P K E	O	12	13th	14th	14th	23	17th	21st	21st	36	14th	..	..
16	N P K E	P	20	21st	19th	17th	22	17th	19th	16th	34	23rd	..	17th

*Remarks.*—E Treatment refers to combination of salts when salts other than N, P<sub>2</sub>O<sub>5</sub> or K<sub>2</sub>O were supplied.

After the rearrangement of pots the solutions containing the various nutrient salts were added. The culture media used was modified by Knop's solution as recommended by Shull and Loomis (1938), and balanced for sand culture work as suggested by McCall (1916). Various salts were replaced according to the scheme given by Shull and Loomis (*loc. cit.*) for excluding N,  $P_2O_5$  or  $K_2O$  as the case may be from the full culture solution. But where only N,  $P_2O_5$  or  $K_2O$  were to be applied separately or in combination, as shown in Table I, the same salts as used for the culture solution, were employed. For the control treatment, distilled water was used every time the solution was applied in the pots.

The plants were allowed to grow till the beginning of May when respiration studies on A and C plants were taken up. Excised shoots constituting the leaf tuft bearing two leaves below the standard (transverse mark) leaf

Fig. 1  
RELATIVE RATE OF RESPIRATION IN  
DIFFERENT CULTURE MEDIA  
(May Series)



were used for the investigation. Method of gaseous exchange was employed for conducting respiration experiments. The apparatus described by Luthra and Cheema (1931) was used for the purpose with the slight modification as given by Khanna and Raheja (1938). This modification consisted in the use of a tower filled with pumice-stone saturated with concentrated

sulphuric acid to free the air of all moisture before entering the respiration chamber. From the  $\text{CO}_2$  evolved, respiration rates were worked out on the basis of per gram fresh weight of material.

The one plant left over was allowed to grow till about middle of November. The respiration rates of excised shoots were worked out as above for each of the single plants in the pots. Almost simultaneously with the above determination, the respiration of whole plants were also determined. Besides measurements of length and maximum width of the last three fully matured leaves were also recorded. Further notes were taken on the leaf colour, nature of the leaf tuft and extent of the leaf tip drying.

### III. EXPERIMENTAL RESULTS

The respiration data obtained during the month of May and November are presented below (Table II), while Fig. 1 in the text shows the relative rate of respiration of plants during May in the various cultural media (Table II).

The plotted points are the mean deviations from the control obtained by summing up all the values that are not asterisked in Table II minus the mean value for control and have been given (Table III, Column 11). It will be noticed that the respiratory activity of plants grown in culture media containing nitrogen salts except in the treatment N.K.E. is low (negative). On the other hand, the application of potassium salts increased, in general, the rate of respiration.  $\text{P}_2\text{O}_5$  alone or in combination with  $\text{K}_2\text{O}$  increased the metabolic activity. Effect of E alone increased the respiration rate (Table III).

It will, however, be observed that the number of observations (Column 3, Table III) for each varied, and there were considerable variations in the respective values of the respiration rate of plants grown in culture media. To obviate the difficulty of unequal numbers, for working out the approximate analysis of variance, 'Effect Values' were derived by the method described in the appendix of the paper. These values have been graphed in Fig. 2.

It is observed that effect of nitrogen, potassium and E in the derived 'Effect Values' was also the same. Nitrogen in every case tended to decrease the rate of metabolism of the plant. The maximum effect of increasing the rate of respiration was indicated by potassium followed by E and least that of phosphorus.

The analysis of variance as worked out for the May series is given below (Table IV).

TABLE II

*Showing respiration of plants during May and November*

Sl. No.	Treatment	Pot No.	Rate of respiration in c.c. per hour per gram fresh weight			
			May A	May C	November B	
1	Control	..	26	0.340	0.274	1.043
			21	0.390*	0.308*	..
			28	..	..	0.871*
			13	0.184	0.254	0.354
			9	0.169	0.178	0.941
2	N	..	35	0.331	0.589	1.024
			1	0.287	0.541	0.836*
			2	0.360	0.289	0.652
3	P	..	30	0.377	0.327	0.490
			7	0.200	0.127	0.608
			24	0.311	0.253	0.389
4	N. P.	..	16	..	..	0.836
			38	0.174	0.284	0.601
			41	1.011	0.404	1.383
5	K	..	19	..	0.449*	0.534*
			3	0.285	0.177	1.285
			32	0.223	0.210	0.831
6	N. K.	..	17	1.02*	..	0.680*
			4	0.651	0.153	1.430
			40	0.386	0.430	0.366
7	P. K.	..	44	1.342	0.269	0.384
			40	0.224	0.315	0.576
			10	0.247	0.141	0.322
8	N. P. K.	..	39	..	..	..
			37	0.367	0.629	1.121
			6	0.275	0.346	0.668
9	E	..	14	..	0.548*	2.680*
			43	0.200	0.319	1.656
			15	0.166	0.202	0.742
10	N. E.	..	25	..	..	0.387*
			8	0.181	0.628	0.904
			45	..	0.114*	1.800*
11	P. E.	..	31	..	..	0.721*
			42	0.314	0.324	0.831
			11	0.204*	..	1.150*
12	N. P. E.	..	47	0.146	0.220	0.252
			5	0.374	1.438	1.349
			27	0.233	0.361	1.166

TABLE II—(Contd.)

Sl. No.	Treatment	Pot No.	Rate of respiration in c.c. per hour per gram fresh weight		
			May A	May C	November B
13	K. E.	29	..	..	1.283*
		18	0.499	0.275	0.761
		33	0.805	0.221	0.792
14	N. K. E.	48	0.280*	..	0.522*
		12	0.423	0.542	1.660
		23	0.706	0.448	0.728
15	P. K. E.	36	..	..	0.788*
		22	0.176*	0.133*	..
		20	0.359	0.144	1.724
16	N. P. K. E.	34	..	0.196*	0.983*

\* These figures were treated as abnormal and were, therefore, not used for interpretation of the data. It will be noticed that they relate to the data obtained with plants which were either one or two in a pot, i.e. all three plants were not there or else in the November series, the plant had dried and therefore the respiration data was omitted.

TABLE III

Showing rate of respiration of plants in the May series

Sl. No.	Treatments	No. of observations	Rate of respiration in c.c.						Mean value	Deviations from the control values	Derived effect values
			No. 1	No. 2	No. 3	No. 4	No. 5	No. 6			
1	2	3	4	5	6	7	8	9	10	11	12
1	Control ..	2	0.340	0.274	..	..	..	..	0.307	..	..
2	N. ..	6	0.184	0.254	0.167	0.178	0.331	0.589	0.284	-0.023	-1.506
3	P. ..	6	0.287	0.360	0.377	0.541	0.289	0.327	0.364	+0.057	-0.190
4	N. P. ..	4	0.200	0.127	0.311	0.253	..	..	0.223	-0.084	-0.304
5	K. ..	4	0.174	0.284	1.011	0.404	..	..	0.468	+0.161	+0.862
6	N. K. ..	4	0.285	0.177	0.223	0.210	..	..	0.224	-0.083	-0.456
7	P. K. ..	6	0.651	0.386	1.342	0.153	0.430	0.269	0.539	+0.232	-0.192
8	N. P. K. ..	4	0.226	0.315	0.247	0.141	..	..	0.232	-0.075	-0.074
9	E. ..	4	0.367	0.629	0.275	0.346	..	..	0.404	+0.097	+0.450
10	N. E. ..	4	0.200	0.319	0.166	0.202	..	..	0.222	-0.085	-0.076
11	P. E. ..	2	0.181	0.628	..	..	..	..	0.405	+0.098	-0.340
12	N. P. E. ..	4	0.314	0.224	0.146	0.220	..	..	0.226	-0.081	+0.058
13	K. E. ..	4	0.374	1.438	0.233	0.361	..	..	0.602	+0.295	+0.292
14	N. K. E. ..	4	0.499	0.275	0.805	0.221	..	..	0.450	+0.143	+0.318
15	P. K. E. ..	4	0.423	0.542	0.706	0.48	..	..	0.530	+0.223	-0.358
16	N. P. K. E. ..	2	0.359	0.144	..	..	..	..	0.252	-0.055	-0.184

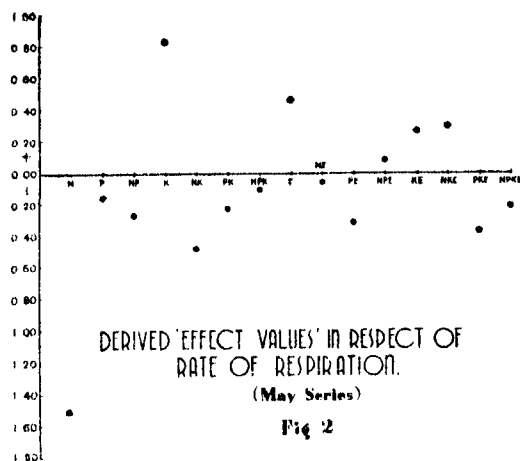
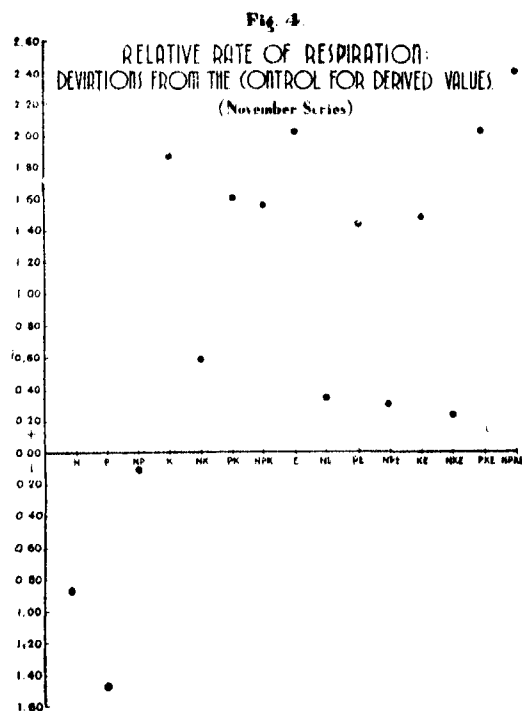
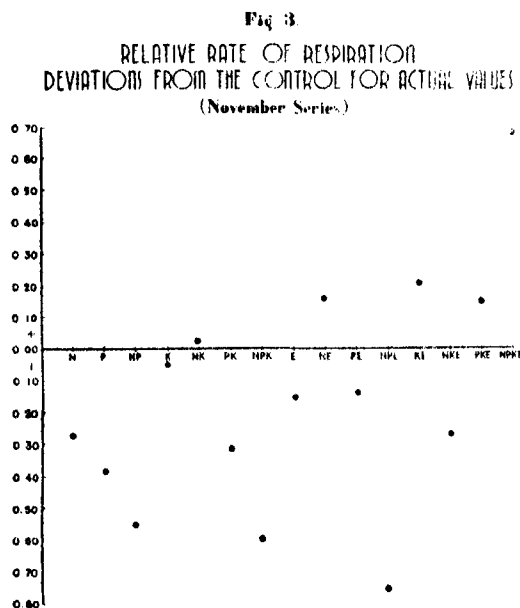


TABLE IV  
*Showing approximate analysis of variance of May series*

Due to	D. F.	S. S.	M. S.	'F' values			
				Observed	Theoretical		
					P-0.20	P-0.05	P-0.01
N	1	0.141752	0.141752	1.799	1.70	4.08	7.31
P	1	0.002256	0.002256	0.029	do	do	do
K	1	0.046443	0.046443	0.590	do	do	do
E	1	0.012656	0.012656	0.161	do	do	do
N. P.	1	0.005776	0.005776	0.073	do	do	do
N. K.	1	0.012906	0.012906	0.164	do	do	do
N. E.	1	0.000361	0.000361	0.005	do	do	do
P. K.	1	0.002304	0.002304	0.029	do	do	do
P. E.	1	0.007225	0.007225	0.092	do	do	do
K. E.	1	0.005329	0.005329	0.068	do	do	do
N. P. K.	1	0.000342	0.000342	0.004	do	do	do
N. P. E.	1	0.000210	0.000210	0.003	do	do	do
N. K. E.	1	0.000323	0.000323	0.080	do	do	do
P. K. E.	1	0.008010	0.008010	0.102	do	do	do
N. P. K. E.	1	0.002116	0.002116	0.027	do	do	do
Error	48	3.781415	0.078779	..	..	..	..
Total	63	4.035514	..	..	..	..	..

From this analysis, it is apparent that nitrogen effect referred to above was the highest though not significant. Since the variations in the respiration rate were very wide, the significance values were tested at 20% level of significance but no effect came out as significant. Wherever nitrogenous salt combined with any other salt or a combination of salts, the effect was to equalise the respiration rate to that of the control series. These interesting results have been discussed later in the text,

The supply of nutrients was continued upto the middle of July and later the plants were starved of their respective nutrient supply with a view to obtain wide differences in the rate of metabolism in various series (Parija and Saran, *loc. cit.*). The results of the respiratory studies conducted in November are given (Table V) in the text. It will be noticed that the number of plants taken up for investigation were different for the different series. Some of these plants had completely died out while others had to be discarded on grounds similar to those reported under the former studies. Fig. 3 illustrates the mean deviations in the respiratory activity from the



control series (Table V, Column 8). The respiratory activity was low when either nitrogen or phosphorus was applied and it was high when potash was given to the plants. The results were not found to exactly correspond with those stated above. Derived values (Fig. 4) were, therefore, worked out by the same method as given in the Appendix.

It is obvious that wherever potassium salt was supplied in nutrient culture media, the rate of katabolic process was high. Application of nitrogen in the form of calcium nitrate  $[Ca(NO_3)_2]$ , however, tended to decrease the rate of respiration except in the combinations N P K and N P K E. Effect of E (combination of minor elements) was to enhance the rate of respi-



TABLE V

*Showing the rate of respiration of plants in the November series*

Sl. No.	Treatments	No. of observations	Rate of respiration in c.c.			Mean value	Deviations from the control	Derived effect values
			1	2	3			
I	2	3	4	5	6	7	8	9
1	Control	1	1.043	..	..	1.043	..	14.439
2	N	3	0.354	0.941	1.024	0.773	-0.270	-0.901
3	P	3	0.836	0.652	0.490	0.659	-0.384	-1.545
4	N. P.	2	0.608	0.389	..	0.498	-0.545	-0.141
5	K.	2	0.601	1.383	..	0.992	-0.051	+1.915
6	N. K.	2	1.285	0.831	..	1.058	+0.015	+0.575
7	P. K.	3	1.430	0.366	0.384	0.727	-0.316	+1.567
8	N. P. K.	2	0.576	0.322	..	0.449	-0.594	+1.475
9	E	2	1.121	0.668	..	0.894	-0.149	+2.041
10	N. E.	2	1.656	0.742	..	1.199	+0.156	+0.385
11	P. E.	1	0.904	..	..	0.904	-0.139	+1.521
12	N. P. E.	2	0.331	..	0.252	0.292	-0.751	+0.329
13	K. E.	2	1.349	1.165	..	1.257	+0.214	+1.409
14	N. K. E.	2	0.761	0.792	..	0.776	-0.267	+0.137
15	P. K. E.	2	1.060	0.728	..	1.194	+0.151	+1.997
16	N.P.K.E.	1	1.724	..	..	1.724	+0.681	+2.381

TABLE VI

*Showing approximate analysis of variance of November series*

Due to	D. F.	S. S.	M. S.	'F' values			
				Observed	Theoretical		
					P-0.20	P-0.05	P-0.01
N	1	0.050738	0.050638	0.217	1.79	4.49	8.53
P	1	0.149189	0.149189	0.638	do	do	do
K	1	0.229201	0.229201	0.980	do	do	do
E	1	0.260355	0.260355	1.114	do	do	do
N. P.	1	0.001242	0.001242	0.005	do	do	do
N. K.	1	0.020664	0.020664	0.088	do	do	do
N. E.	1	0.009264	0.009264	0.039	do	do	do
P. K.	1	0.153468	0.153468	0.656	do	do	do
P. E.	1	0.144590	0.144590	0.618	do	do	do
K. E.	1	0.124080	0.124080	0.531	do	do	do
N. P. K.	1	0.135977	0.135977	0.581	do	do	do
N. P. E.	1	0.008765	0.008765	0.029	do	do	do
N. K. E.	1	0.001173	0.001173	0.005	do	do	do
P. K. E.	1	0.249251	0.249251	1.066	do	do	do
N. P. K. E.	1	0.354323	0.354323	1.616	do	do	do
Error	16	3.740210	0.233763	..	..	..	..
Total	31	5.630490	..	..	..	..	..

ration. Analysis of variance (Table VI) revealed that the variations were higher for E, P K E and N P K E. The effects for P K, N P K and K E were also fairly high. Though none of the effects is significant, it will be noticed in the above combinations that both in the absence and presence of E, the effect of K, P K and N P K was more or less similar. Evidently K being common to all, its effect on respiration was throughout very marked. These differences in the effect of nutrient salts on the rate of respiration of foliage have been discussed later in the text.

*B. Dry weight data.*—Dry weight studies were carried out for the November series and the results (Table VII) when arranged in descending order of yield were N K E, N P K E, N P K, N P E, N P, N, N E, P E, P K E, P, K E, N K, K, P K and E.

TABLE VII

*Showing dry matter percentage of plants for the November series*

Sl. No.	Treatments	Dry matter of plants (gm.)				Mean	Deviations from the control
		No. 1	No. 2	No. 3	Total		
1	Control ..	12.04	13.96*	23.60*	12.04	12.04	..
2	N ..	26.64	27.64	27.00	81.28	27.09	+15.05
3	P ..	13.79*	19.33	15.20	34.53	17.26	+ 5.22
4	N. P. ..	30.13	29.04	64.06*	64.17	32.09	+20.05
5	K ..	10.51	12.89	11.30*	23.40	11.70	- 0.34
6	N. K. ..	13.54	20.87*	14.64	28.18	14.09	+ 2.05
7	P. K. ..	12.96	10.24	10.62	33.82	11.27	- 0.77
8	N. P. K. ..	43.74	39.17	52.67*	82.91	41.46	+29.42
9	E ..	7.74	11.80*	10.19	17.93	8.97	- 3.07
10	N. E. ..	27.03	81.93*	23.94	50.97	25.49	+13.45
11	P. E. ..	19.58	31.61*	17.78*	19.58	19.58	+ 7.54
12	N. P. E. ..	36.90*	39.63	34.78	74.41	37.21	+25.17
13	K. E. ..	16.04	15.47	21.24*	31.51	15.76	+ 3.72
14	N. K. E. ..	44.61	45.13	55.58*	80.74	44.87	+32.83
15	P. K. E. ..	18.05	16.58	30.64*	34.63	17.32	+ 5.28
16	N. P. K. E. ..	43.69	43.99*	37.99*	43.69	43.61	+31.57

\* Dry matter values for those plants of which the results of respiration were omitted have not been taken into consideration while working out the mean.

From the order stated above, it is evident that the application of nitrogen throughout was more effective than that of either phosphorus or potash in increasing the accumulation of dry matter in the plant. Except in the treatment N K E the combined effect of N P was much more than that of P or N alone. Effect of K, unless it was in combination with N P, was always to depress the yield factor. The relationship between the respiratory process and the yield factor has been discussed subsequently.

## IV. DISCUSSION AND RESULTS

According to the fourth principle of agro-biology, a certain definite amount or intensity of growth factor produces a certain definite result (Willcox, 1930). In other words, the effect is a quantitative one. In an ensemble of growth factors also, the effect of any one of them is additive or cumulative. In the experiments reported above this has been clearly brought out in both the respiratory activity of plants carried out in May and that completed in November. At both the periods, the specific effect of nitrogen was to reduce the rate of respiration while addition of K, or E, except in combination with N P, was always to enhance the rate of respiration. It appears that nitrogen by virtue of its being a rapidly absorbed anion brings about more effective equilibrium in the rate of metabolism, that is to say, the accumulation of substances with nitrogen is higher and, therefore, the carbon dioxide output per unit of accumulated substance is low. No other substance reduces the respiratory quotient of the plant like nitrogen. In May series particularly, the effect of the presence of phosphorous salts, was to increase the rate of katabolic process. Combined with nitrogenous salts it was more effective in reducing the rate of respiration. Potassium salts in conjunction with N or P showed the least effect in reducing the rate of katabolism. But when all the big three—N, P and K combined, the effect was to reduce the rate of respiration. The combined effect of the salts other than those having nitrogen, phosphorus or potash was to enhance the breakdown of the metabolisable material and this was irrespective of the big three elements. The age of the plant did not matter in this respect because the specific effect was identical in both the May and the November series.

In the case of dry matter output, nitrogen in combination with the rest was most effective; the next in order being the combination of nitrogen and phosphorus. Effect of potash alone or in separate combination with nitrogen, phosphorus, nitrogen plus E, and phosphorus plus E was rather deleterious to the accumulation of dry matter.

From the results of the respiratory activity of plants and the accumulation of assimilated products, one may infer that potash is the first limiting factor of the three big ones, for it is the earliest to show its deleterious effect under the conditions of the experiment described above. The antagonism of salt exists so long as it does not combine with nitrogen and phosphorus but this is not less apparent because of the presence of high or low concentration of growth factors. This is clear from the similarity of the results in respect of respiration obtained from May series, when plants were maintained at the normal salt requirement, and from the November series, when the plants had been kept starved.

## V. SUMMARY

Investigations on metabolic activity of plants were carried out in sand culture to study the specific influence of nitrogen, phosphorus, potash and combination of other minor elements, alone and in combination with one another. Respiration experiments were performed on plants grown upto November. The application of nutrient supply was stopped in mid-July when plants were starved with a view to study the antagonistic effect under normal and abnormal conditions of growth. The dry matter of plants harvested in November was also estimated. The results arrived at were as follows:

(i) Nitrogen decreased the rate of respiration. It was most effective in reducing the rate of respiration when it was applied in combination with all the other elements.

(ii) Effect of potash was just the opposite to that of nitrogen. It enhanced the rate of respiration unless it was present in combination with N P.

(iii) Phosphorus alone increased the rate of metabolism. But in combination with nitrogen it was very effective in reducing the rate of respiration and in combination with potash in keeping up a higher rate of respiration.

(iv) Effect of the combination of salts of the minor elements alone was to increase the rate of katabolic processes. In combination with nitrogen, phosphorus or potash, this effect was much less apparent.

(v) Nitrogen was most effective in the accumulation of dry matter; phosphorus was the next best while neither potash nor combination of other salts of minor elements effected any increase in the dry matter.

(vi) Potash has been shown as the first limiting factor as it is the earliest to show adverse effect on the respiratory process and it maintains its antagonism so long it does not combine with nitrogen and phosphorus together.

(vii) The antagonistic effect on respiratory process is maintained both under normal and abnormal conditions (starvation) of nutrient supply.

## VI. ACKNOWLEDGEMENTS

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FIG. 1

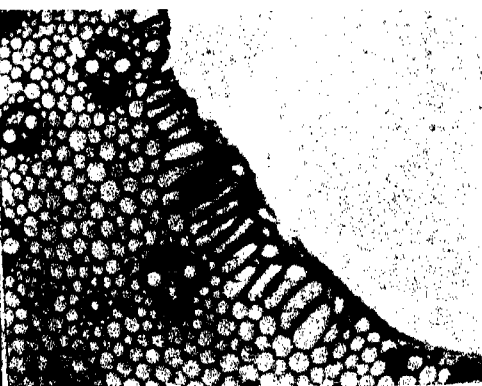


FIG. 4

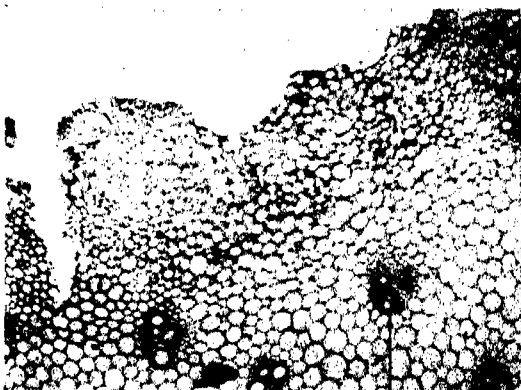


FIG. 2

*a*

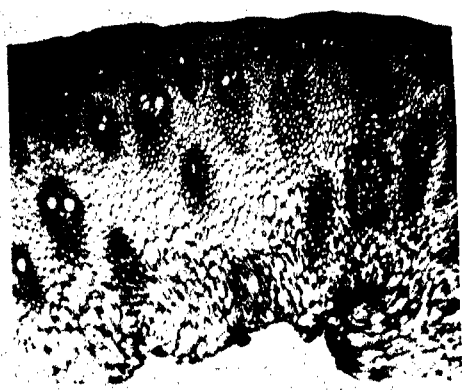


FIG. 5

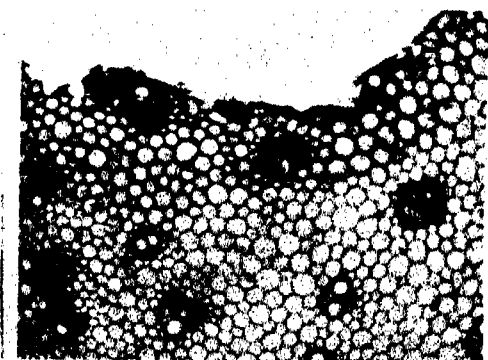


FIG. 3

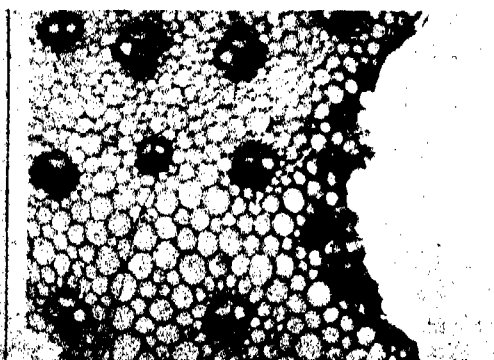


FIG. 6



# APPENDIX

The experiment consists of four treatments (N, P, K and E) at two levels each, and is therefore of the type  $2^4$ . Presence and absence of a treatment are its two levels. Denoting the presence of a treatment by the suffix 1 and its absence by zero, the main effects and interactions may be represented as follows:

$$\begin{aligned} N &= \frac{1}{8} (N_1 - N_0) (P_1 - P_0) (K_1 + K_0) (E_1 + E_0) \\ NP &= \frac{1}{8} (N_1 - N_0) (P_1 - P_0) (K_1 + K_0) (E_1 + E_0) \\ NPK &= \frac{1}{8} (N_1 - N_0) (P_1 - P_0) (K_1 - K_0) (E_1 + E_0) \\ NPK E &= \frac{1}{8} (N_1 - N_0) (P_1 - P_0) (K_1 - K_0) (E_1 - E_0) \\ &\dots\dots\dots \\ &\dots\dots\dots \end{aligned}$$

and so on for the other main effects and interactions.

Each of the above expressions, which stands either for a main effect or an interaction represents 1 degree of freedom, and when expanded, will consist of 16 terms, 8 of which are positive and 8 negative and the estimate of a main effect or an interaction will be obtained by substituting the value (rate of respiration) for each term in the expression obtained after expansion.

In comparing these effects amongst themselves, the multiplying factor ' $\frac{1}{8}$ ' has been omitted throughout and "Effect Values" have been derived by substituting for each term (e.g.,  $N_1 P_1 K_0 E_0$ ) in the expression, the mean value of respiration for all the pots occurring under the treatment combination ( $N_1 P_1 K_0 E_0$ ).

In getting the sum of squares due to any of these main effects or interactions in the *approximate* analysis of variance, the arithmetical value of the expression (effect value) corresponding to the relevant effect has been squared and then divided by 16. 16 has been used as the dividing factor, because each expression has been evaluated from 16 averages.



**BOMOLOCHUS ACUTA N.SP., A COPEPOD  
PARASITIC ON THE GILLS OF  
DUSSUMIERIA ACUTA**

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(Communicated by Prof. S. G. M. Ramanujam, M.A., Ph.D., F.A.Sc.)

A SINGLE mature female specimen of this Ergasilid parasite was found attached to the gills of the Madras Rainbow Sardine *Dussumieria acuta*. A full description is given here since our knowledge of copepods parasitic on Indian fishes is very scanty as indicated in a previous paper (6) and since the Ergasilidæ which are not far removed from the free living forms consist of only a few genera and species and the earlier descriptions are far from accurate. The parasite measures 2.72 mm. long and 0.75 mm. across the cephalothorax, the widest part of the body. The cylindrical egg sacs, 1.32 mm. long, form a conspicuous feature. Behind the semicircular broad cephalothorax the rest of the thoracic segments succeed in decreasing width and end in a narrow abdomen lying between the two long egg sacs. The cephalothoracic shield or carapace is not merely convex above, concave below, but has an edge fringed by a softer thinner extension of the ventral plate, helping the adhesion of the parasite by suction. This is facilitated further by a deeper caving in of the ventral surface brought about by the folding dorsalwards and forwards of the cephalon just behind the maxillipedes and in front of the first swimming leg of the thorax. As a result, the cephalothorax serves as a large sucker bounded in front by the frontal area, on the sides by the thick edge of the carapace and behind by the first swimming legs. The cephalothorax is semicircular in outline with the straight posterior side 0.75 mm. wide, fused to the first free thoracic segment along the whole width. Anteriorly the frontal region is marked by a deep median notch to the bottom of which is attached a frontal plate. This elliptical plate is folded transversely in the middle in such a way that the front half hangs down. The anterior (i.e., the lower) margin bears two processes with swollen bases and acuminate tips. The dorsal convex surface of the carapace is marked by a median furrow starting close to the hind border of the cephalothorax. As it runs forwards, the furrow widens into a valley flanked by two ridges which end in two conspicuous prominences one on either side

of the median notch containing the frontal plate. In the anterior region of the valley just behind the frontal lobe is located the median eye which recalls strongly the median eye of free living copepods. It is of three parts, two lateral and one anterior and median. In the vicinity of the eye, the carapace is marked by several denticles of varied size and form.

*Appendages.*—The cephalothorax bears the six appendages of the cephalon and that of the first thoracic segment fused with the head. As

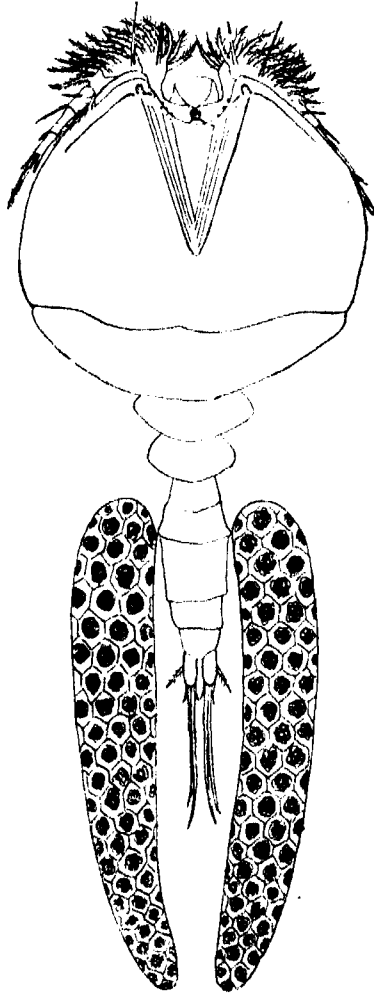


FIG. 1. *Bomolochus acuta* n. sp. Dorsal view of female  $\times 63$ . (Shown without thoracic appendages).

there are many errors in the earlier descriptions of Bassett Smith, Scott, Brian and others, occasioned by defective observation and interpretation of

these appendages, the author of the present paper has adhered to Wilson's descriptions. The first antennæ are large and setose. Each consists of seven segments of which four form the proximal half and three form the distal part of the appendage. The first or basal joint widens anteriorly and bears two plumose setæ, the second joint, which is more dorsal, is bent outwards in such a way that the antennæ projects laterally sweeping round the

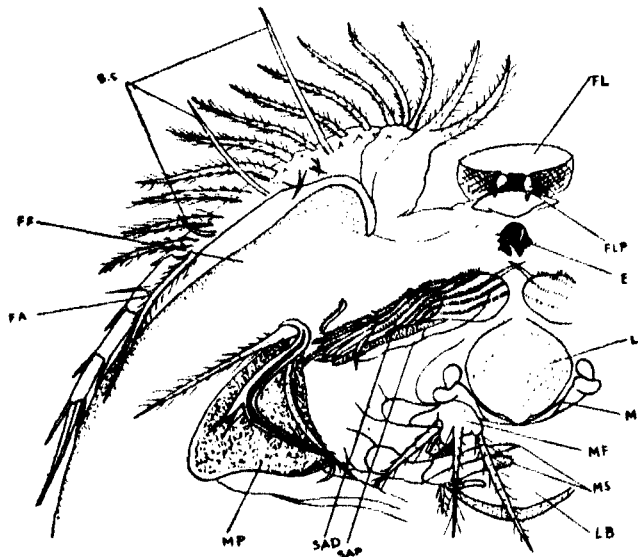


FIG. 2. *Bomolochus acuta* n. sp. Ventral view of head appendages  $\times 150$ .

E.	Median eye.	M.	Mandible.
F.E.	Flexible flange of ventral plate.	M.F.	First maxilla.
F.A.	First antenna.	M.P.	Maxillipede.
F.L.	Frontal lobe.	M.S.	Second maxilla.
F.L.P.	Frontal lobe processes.	S.A.D.	Second antenna distal part.
L.	Labrum.	S.A.P.	Second antenna proximal part.
L.B.	Labium.	S.S.	Sensory spines.

anterior margin of the carapace. This joint, as well as the third and fourth joints, bear three simple sensory spines of varying lengths as well as eleven long plumose setæ on the anterior aspect of the appendage. Each plumose seta projects forwards and curves outwards to its sharp tip. In addition to these the fourth segment bears posteriorly on its outer edge, a long plumose seta. The fifth, sixth and seventh joints form the more slender, less armed distal part of the antenna. Each joint is of a tapering form and bears on its outer edge two short horizontal spines. The terminal joint bears two long setæ set close together. The second antenna is of two halves hinged and folded. The proximal two jointed part is attached behind the

base of the first antenna and is directed inwards so that the distal part is folded outwards. This part also consists of two joints of which one forms the bend or hinge. It is swollen, rounded and bears a short slender spine directed obliquely forwards and inwards. The fourth joint consists of a short cylindrical body to which a number of slender strips and about five long setæ hooked at their distal sharp tips are attached. These slender chitinous strips which are fused spiral-wise on the joint bear numerous teeth

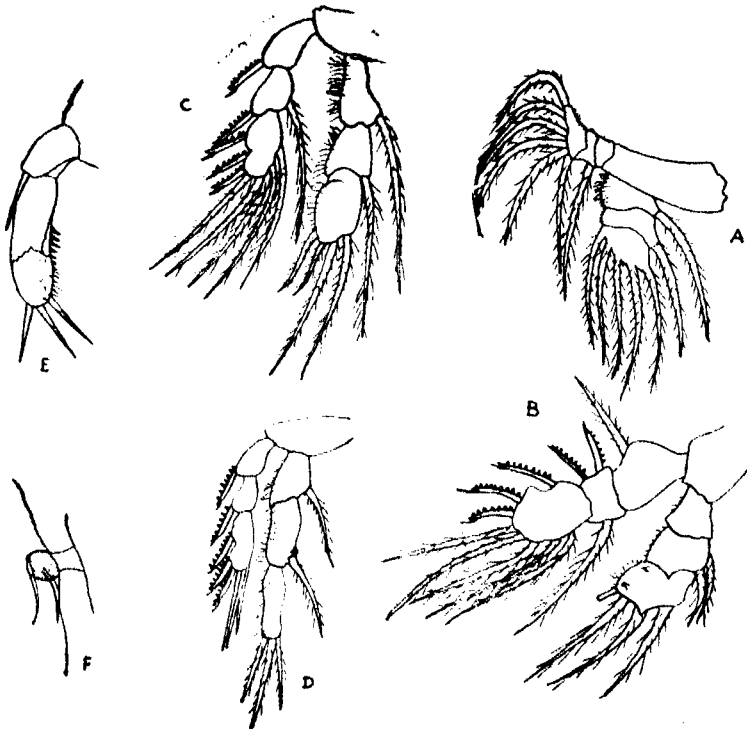


FIG. 3. *Bomolochus acuta* n. sp. Thoracic appendages (not drawn to scale).

- |                    |                               |
|--------------------|-------------------------------|
| A. First thoracic  | D. Fourth thoracic            |
| B. Second thoracic | E. Fifth thoracic             |
| C. Third thoracic  | F. Sixth thoracic (vestigial) |

closely packed together and provide rough surfaces which help adhesion. Between the rounded inner ends of the second antennæ, the obovate labrum is attached. On either side of this upper lip in front of the labium are arranged three appendages on each side. Of these *the mandibles* are most anterior and closest to the labrum. It consists of a basal joint bent backwards, a middle joint with two rounded articular knobs at the base, as well as a distal cylindrical tapering portion. There is no palp and the terminal

joint is destitute of setæ. *The first maxillæ* are three jointed; a stout cylindrical muscular portion directed inwards, is followed by a similar second joint. The third part is bulbous and bears four long stiff plumose setæ directed backwards in different directions as well as a short setose palp-like body. *The second maxilla* consists of a two articulated basal part and a bipartite distal part. The two rami are directed medially. One of these is sharp pointed while the other is blunt tipped, both being setose. *The maxillipedes* are three jointed. The basal joint is attached to the ventral surface of head behind the second maxillæ but is turned outside mouth parts in such a way that the rest of the appendage appears not only outside but also anterior to the other mouth parts. The second joint is roughly triangular in form, the inner proximal corner of which bears a slender sharp claw. The third joint is formed by a sigmoid curved stout claw directed backwards. This claw bears two sharp teeth at its second bend and ends in a sharp point. To the base of the claw are attached two plumose setæ one directed outwards and the other inwards.

*Thoracic appendages.*—The first are flattened and biramous. The basal joint is long. The exopod is three jointed and bears seven long plumose setæ; one on the second and six on the last joint. The endopod is also three jointed, the first and second joints bear one plumose seta each, while the third bears five plumose setæ. The second, third and fourth pairs of swimming feet also are biramous but are less flattened. In each leg the basal joint is single and supports both the exopod and endopod. The endopods are clearly three jointed while the exopods are really four jointed but, owing to the fusion of the last two joints, appear three articulated. The joints are covered with hairs and bear a number of spines and setæ which are distributed as follows:—

II Endopod 0-1, 2-2, 1-3; II Exopod 0-1, 2-1, 3-6; III Endopod 0-1, 0-1, 2-2; III Exopod 1-0, 1-1, 3-5; IV Endopod 0-1, 0-1, 2-1; IV Exopod 1-0, 1-0, 3-3.

The fifth leg differs from the foregoing four in being uniramous as in all Ergasilids. They are smaller but are clearly three jointed. A well marked basal joint bears a long slender spine at its outer distal margin. The second joint is twice as long and bears a number of teeth on its inner aspect and the third joint is short, rounded, setose and bears three spines at its distal tip. The sixth leg is vestigial, consisting of a papilla and two slender spines.

Of the six thoracic segments the first has fused with the head to form the cephalothorax, the remaining five being distinct and free. The first of these is as broad as the cephalothorax but has a markedly convex hinder border

so that the next segment which is a third in width is articulated to its narrow posterior hump. The third segment resembles the second in being ellipsoidal but smaller being only two-thirds in width. The fourth segment is much narrower, being only half the breadth of the third. But its posterior edge being wider leads up to the slightly wider, genital segment. To the sides of this segment are attached the two long cigar-shaped egg sacs 1.32 mm. in length. These egg sacs are broadest at the anterior end being 0.17 mm. and taper posteriorly to a blunt point. Each egg sac contains about six to seven longitudinal rows of eggs, each row consisting of eighteen to twenty eggs.

*The abdomen*:—is three segmented and is of such a tapering form that the third segment narrows to nearly half the width of the first. Two anal laminae, each bearing a short slender plumose seta on the outer side and a couple of spines on its distal margin are attached to the last segment. To the tip of each lamina are attached two long plumose setae as long as the abdomen and anal laminae put together. Of the two, the inner is slightly longer.

*Systematic position*.—This parasite clearly belongs to the genus *Bomolochus* (of the sub-family Bomolochinae) since all the thoracic segments except the first are distinct and freely movable and the basal joints of the first antennae are enlarged, flattened and densely armed. According to Wilson's artificial key, the maxillipedes being turned forward outside the other mouth parts, appears to be significant. The terminal claw on maxillipedes being sigmoid in shape and bearing two more teeth, the exopod of the first swimming leg having three joints of which the two terminal joints bearing plumose setae and the free thoracic segments being of decreasing width, emphasise the resemblance to *B. exilipes* n. sp. Wilson. The present form, however, differs from this species in having only two plumose setae (and not three) on the maxillipedes; the first maxilla having four plumose setae (and not three); the first antenna being indistinctly segmented into seven and reaching far beyond the lateral margins; and the second antenna having shorter claws on its inner elbow. A comparison with forms hitherto recorded from the Indo-tropical area is made difficult owing to the incorrect descriptions and inaccurate sketches given by earlier authors. Of the species recorded from the Indo-tropical waters, the following eight species have been compared:—(1) *B. gracilis* found on *Zygæna malleus* Java (Heller, 1865), (2) *B. tetrodontis* found on *Tetrodon oblongus* Bombay (Bassett Smith, 1898), (3) *B. chaetoessi* found on *Chetossus* sp. East Indies (Kroyer, 1863), (5) *B. unicirrus* found on *Amphisile scutata* Ceylon (Brian, 1902), (6) *B. megaceros* found on *Stromateus cinereus* Bombay, Colombo and on *Caranx djedaba*

Aden (Bassett Smith, 1898 and Heller, 1865), (7) *B. tricerus* found on *Stromateus cinereus* Bombay (Bassett Smith, 1898), (8) *B. denticulatus* found on *Sphyræna jello* and *Hemirhampus far*. Of these two have been included by Wilson in a new genus *Irodes* the next three species have been removed to the new genus *Artocolax* (Wilson). The remaining three species differ from the present species in the following features. *B. denticulatus* differs in having the second free thoracic segment globose, longer than the first and bending the body at regular right angles; second antennæ having three terminal spines; the exopod of first leg being two jointed; the body being 3-4 mm. long; the egg sacs being as long as the whole animal and the frontal plate being semilunar, bearing two pedicles. *B. megacerus* is distinguished from the present species in being 4 mm. long; the first antenna having thirteen plumose setæ and three spines; the second antennæ bearing four spines; the sigmoid claw of the maxillipede bearing a single tooth; the second free thoracic segment being as broad as the first free thoracic segment; and in the number and distribution of the denticulate spines, simple spines and plumose setæ. *B. tricerus* differs from the present form in possessing a very long pointed rostrum (not shown in figure); first swimming leg having a single jointed exopod bearing five plumose setæ and an endopod consisting of two joints; the second, third and fourth thoracic legs having no denticulate setæ and the anal lamina bearing a single strong bristle, two short lateral hairs, and a minute hair on outer border.

The present species can be distinguished from others in the following features. All free segments of the thorax except the first being distinct and uniform; a folded rostral plate bearing two processes; the first antenna having seven joints; the maxillipede bearing a sigmoid claw with two teeth and two plumose setæ; the exopod of second, third and fourth legs bearing denticulate spines, the exopod of first swimming leg being three jointed; the second antenna bearing five hooked spines; Hence the present species is described as a new species *Bomolochus acuta*. The type form will be lodged in the Indian Museum, Benares.

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# AMIDE SYNTHESIS IN PLANTS

## Part III. Urea Formation in Seedlings

BY M. DAMODARAN, F.A.Sc., AND T. R. VENKATESAN

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Received November 5, 1947

It was pointed out in the last paper in this series (Damodaran, *et al.*, 1946) that there were reasons for suspecting the formation, during seed germination and seedling growth, of a third amide in addition to asparagine and glutamine. Analysis of the changes taking place in amino-acids and amides in three species of germinating seedlings showed, in the first place, that the increase in the amide nitrogen in the non-protein fraction of the seedling extract was greater than could be accounted for by the increase in glutamine and asparagine; and secondly that in *Phaseolus mungo* and *Dolichos biflorus* in the later stages of seedling growth amide nitrogen was markedly in excess of the total dicarboxylic acids present. As urea has been shown to be widely distributed in fungi as well as in the higher plants (Wehmer, 1933) it was considered possible that this substance might be formed in seed germination also along with asparagine and glutamine.

In fungi urea is formed according to Iwanoff (1924, 1927) from ammonia and its function is to serve as a nitrogen reserve in the fruiting bodies. Klein and Taubock (1932) who studied the formation of urea in the higher plants were of opinion that it was derived from the hydrolysis of arginine and arginine-like compounds by the action of arginase. It was decided to test this hypothesis by carrying out in the course of the analysis described in the last paper simultaneous estimations of the quantities of urea and arginase present in the seedlings. As it has been shown (Damodaran and Sivaramakrishnan, 1937) that many leguminous seeds contain urease it was necessary to determine the amounts of this enzyme also as the quantity of urea present at any stage would represent the balance of the activities of arginase which hydrolyses arginine to give ornithine and urea and of urease which hydrolyses urea to carbon dioxide and ammonia.

## EXPERIMENTAL

The present experiments were confined to *Dolichos biflorus* and *Phaseolus mungo* as these two were the seedlings which showed the greatest disparity between amide nitrogen and dicarboxylic acid content. The procedure with regard to germination of seedlings, preparation of samples for analysis and analytical methods for amino-acids and amides have been

described in the previous paper. Urea estimations were carried out every three days and urease and arginase activity every other day. The analyses of urea, urease and arginase were all reduced to the determination of ammonia the latter being carried out by the elegant micro-diffusion technique of Conway (1939).

*Urea.*—Urea estimations were made in the deproteinised aqueous extract of the seedlings prepared, as described previously, by removing the proteins by heat coagulation. Water melon urease prepared according to Damodaran and Sivaramakrishnan (1937) was used for the decomposition of urea. 1 ml. of standard N/70 sulphuric acid was pipetted out into the middle chamber of Conway's unit and a drop of Tashiro's indicator added. 1 ml. of the test solution was placed in the outside chamber followed by 0.5 ml. of urease (10% suspension in M/15 phosphate buffer at pH 7). The units after being closed airtight with discs smeared with grease (a mixture of paraffin and vaseline) were rotated slowly a few times to mix the enzyme and substrate solution completely and incubated at 37° for decomposition of urea. After 20 minutes 1 ml. of saturated potassium carbonate was added to the outer chamber, the solutions in the outside chamber mixed by gentle rotation as before and the apparatus left again in the incubator for two hours for absorption of ammonia by the acid. Excess of acid in the middle chamber was then titrated against CO<sub>2</sub>-free N/70 sodium hydroxide using a Conway micro-burette. Preformed ammonia was determined in two control experiments in which the outer chamber of the Conway units contained (1) the enzyme urease only and (2) the solution under test only. The sum of the ammonia formed in the two determinations was deducted from that found in the experimental units. At least three units were used for each solution and the estimations discarded if not closely concordant.

*Urease and arginase.*—For preparation of extracts for determination of enzyme activities a constant number of uniformly grown healthy seedlings (usually thirty) was washed after removal of testa and after being lightly dried on filter-paper, thoroughly ground in an ice-cooled granite mortar. For urease estimations the macerate was brought to pH 7 with M/4 phosphate buffer and then made up to 25 ml. with M/15 phosphate buffer. For estimation of arginase the macerate was brought to pH 9.4 by gradual addition of 0.1 N sodium hydroxide and then made up to volume (25 ml.) with glycine-sodium hydroxide buffer of pH 9.4. 5 ml. aliquots were taken for estimation of enzyme activity.

Urease activity determination was carried out by taking 5 ml. of the suspension at pH 7, 5 ml. of urea solution (M/2 when activity was high and

M/10 in other cases) and phosphate buffer at pH 7 to make upto 25 ml. and allowing them to react at 37° for exactly twenty minutes after which period the enzyme was inactivated by the addition of 5 ml. of N-sulphuric acid and keeping at 80° for ten minutes. The solution was made upto volume and ammonia determined on an aliquot in the Conway apparatus. A control experiment using boiled seedling extract was carried out at the same time. The total N in the suspension was determined in each case and urease activity expressed as the number of mgm. of urea N decomposed per 100 mgm. total nitrogen in 20 minutes.

For determination of arginase activity the following procedure was adopted. 5 ml. of the suspension under test, 5 ml. of M/100 arginine solution (shown by preliminary experiments to provide excess substrate) both at pH 9.4 were mixed and made up to 25 ml. with sodium hydroxide-glycine buffer at pH 9.4. The reaction mixture was allowed to stand at 37° for three hours under a layer of toluene. At the end of the period the enzyme was inactivated as before by keeping at an acid reaction at 80° for 10 minutes. The solution was then brought to pH 7 by addition of N-sodium hydroxide and urea in an aliquot determined by the method described above. A control was conducted as usual with boiled enzyme. Arginase activity was expressed as the number of mgm. urea N formed from arginine in three hours.

## RESULTS

TABLE I

### *Dolichos biflorus* seedlings

Values expressed as percentage of N of seedlings: Enzyme units per 100 mgm. of seedling N

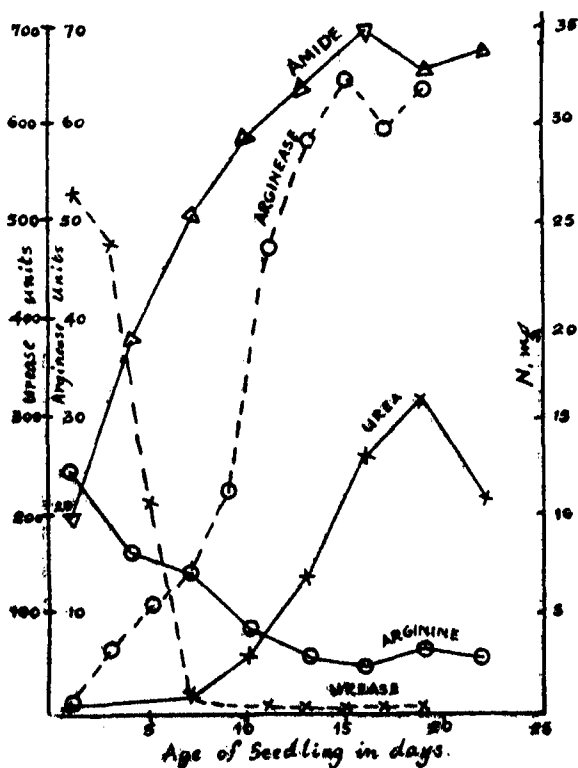
Age of seedlings in days	1	3	4	5	7	9	10	11	13	15	16	17	19	22
Amide ..	9.8		18.9		25.1		29.2		31.8		34.8		32.7	33.8
Dicarboxylic acids ..	21.4		24.7		25.6		25.6		24.6		22.9		23.9	23.4
Excess amide over dicarboxylic acids							3.6		7.2		11.9		8.8	10.4
Urea ..	0.3		0.5		0.8		2.8		6.9		13.0		16.0	11.0
Arginine ..	12.4		8.2		7.1		4.3		2.8		2.3		3.3	2.6
Fall in arginine ..			4.2		5.3		8.1		9.6		10.1		9.1	9.8
Arginase units ..	0.9	6.5		10.9	14.1	22.7		47.8	58.1	64.4		59.3	63.6	
Urease units ..	527	477		215	13.7	4.9		4.7	2.9	2.2		3.8	3.4	

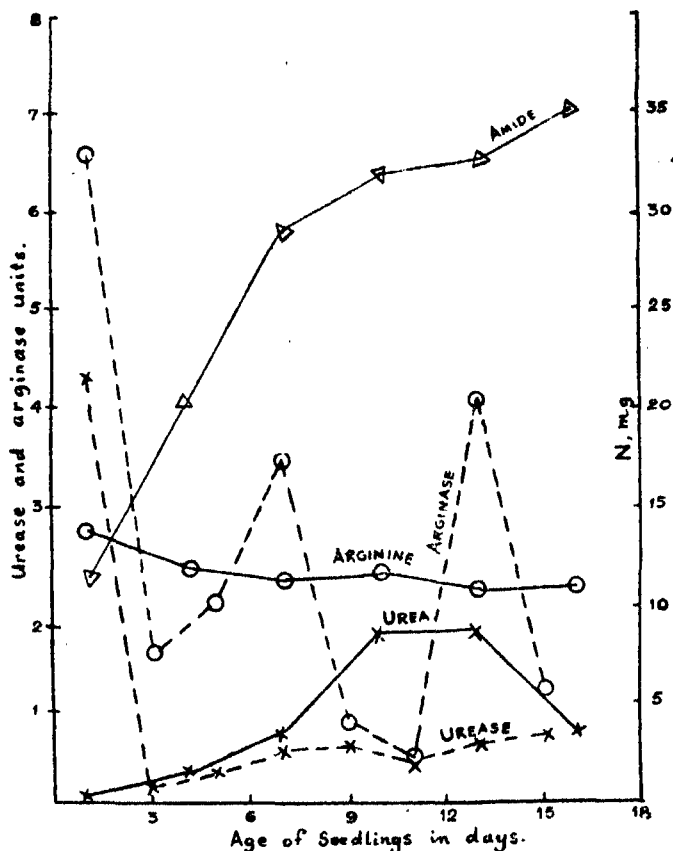
TABLE II

## Phaseolus mungo seedlings

Values expressed as percentage of N of seedlings: Enzyme, units per 100 mgm. of seedling N

Age of seedlings in days	1	3	4	5	7	9	10	11	13	15	16
Amide ..	11.2		20.3		29.0		31.7		32.5		35.1
Dicarboxylic acids ..	23.1		28.0		32.4		28.4		28.5		29.3
Excess amide over dicarboxylic acids							3.3		4.0		5.8
Urea ..	0.2		1.3		3.4		8.5		8.8		3.5
Arginine ..	13.9		12.0		11.3		11.6		10.6		10.9
Fall in arginine ..			1.9		2.6		2.3		3.3		3.0
Arginase units ..	6.6	1.5		2.0	3.5	0.8		0.4	4.1	1.1	
Urease units ..	4.3	0.14		0.27	0.5	0.56		0.35	0.56	0.63	

FIG. 1. *Dolichos biflorus*

FIG. 2. *Phaseolus mungo*

## DISCUSSION

It is clear from the results obtained that formation of urea is one of the important chemical changes taking place during seed germination and reaches quite considerable proportions. Thus in the nineteen-day old seedlings of *Dolichos biflorus* the amide nitrogen is 32.7% and the urea 16.0% or nearly half of the total amide present. In *Phaseolus mungo* urea production reaches its maximum on the 13th day. At this stage amide nitrogen is 32.5% and urea 8.6% or 28% of the total amide. The quantity of urea produced is more than sufficient to account for the excess of amides over dicarboxylic amino-acids and explains this discrepancy.

The origin of the urea is, however, not so clear. In *D. biflorus* there is reason to conclude that arginine is the precursor of at least part of the urea found. There is a considerable and rapid decline in the quantity of the arginine present while simultaneously there is a parallel change in enzyme

activity in the seedling, the urease activity showing a sharp fall and the arginase activity a steady increase (Fig. 1). But as only half the arginine N can appear as urea the fall in arginine, as the figures in Table I show, is insufficient to account for the whole of the urea formed. In *P. mungo* the proportion of the urea that could be formed from the arginine that has disappeared is still smaller. There is no great decrease in the arginine content of the seedling throughout the experimental period; the arginase activity is also low and shows no such steady rise as in *D. biflorus* (Fig. 2). The hydrolysis of arginine cannot therefore account for more than a fraction of the urea formed. Klein and Taubock were of opinion that urea arose from arginine-like compounds such as canavanine. An examination of several leguminous seeds made in this laboratory for canavanase in connection with a former investigation (Damodaran and Narayanan, 1940) however, gave no indication for the presence of this amino-acid in the seed reserves of either *P. mungo* or *D. biflorus*. While the presence of other unknown precursors of urea cannot be excluded a more plausible hypothesis for the formation of urea (other than that arising from arginine) would appear to be by synthesis from ammonia and carbon dioxide either directly by the action of urease or indirectly *via* the Krebs ornithine cycle. Further experimentation is required to test the validity of any such hypothesis.

#### SUMMARY

It has been demonstrated that the formation of urea is one of the important biochemical changes taking place in the course of protein regeneration in seedlings. The quantities of urea formed in *Dolichos biflorus* and *Phaseolus mungo* represent a considerable proportion of the amides formed in these seedlings and explain the observation previously reported that amides are in excess of the dicarboxylic acids.

From the quantitative relations between the changes in urea, urease, arginine and arginase it is inferred that at least in *Dolichos biflorus* a part but not the whole of the urea arises from the hydrolysis of arginine. It is, however, necessary to assume the existence both in *Dolichos biflorus* and *Phaseolus mungo* of an additional mechanism responsible for urea formation.

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## ERRATA

Vol. XXVI, No. 4, Section B, October 1947

On Page 145, first line, *H. cannabinos* should read *H. cannabinus*.

In the list of References against 15, read Thomas, Ramakrishnan, Soumini and Balakrishnan, *Proc. Ind. Acad. Sci.*, (B), 1947, **26**, 147-163 instead of *Proc. Ind. Acad. Sci.*, (B), 1947, **25** (in Press).





## ADDITIONS TO FUNGI OF MADRAS—IV\*

BY T. S. RAMAKRISHNAN AND K. RAMAKRISHNAN

(Mycology Section, Agricultural Research Institute, Coimbatore)

Received September 10, 1947

(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

### (16) *Bulgariastrum tumifaciens* Ramakrishnan T. S. and K. sp. nov.

On pedicels and young fruits producing dark gall-like outgrowths; on leaves forming thickened, brown, isolated to confluent spots; *apothecia*, amphigenous on the leaves, clustered, sub-turbinate, black, disc-shaped, slightly concave at the top, with short stout stalks,  $400-700\mu$  in diameter; *asci* cylindric to clavate, narrowed at the base, hyaline  $80 \times 12\mu$  ( $65-115 \times 10-17\mu$ ), 8-spored, paraphysate, paraphyses branched at the tip, forming thick-walled dark brown cells which become compacted into a dark epithecium; *ascospores* elliptical, two-celled, constricted at the septum, hyaline,  $13 \times 5.4\mu$  ( $10-19 \times 4.8-9.6\mu$ ), more or less uniseriate; *pycnidia* mixed with the apothecia more numerous on the galls on the inflorescence, often developing on the stalks of the apothecium, flattened, immersed in the stroma, or on the surface, covered by arching, black layers of cells which rupture easily and expose the pycnidiospores; sometimes the ruptured pycnidia resemble acervuli; *pycnidiospores* produced on short stalks, 2-celled, fusiform to clavate, light olive, lower cell usually more elongated than the upper cell,  $18 \times 7\mu$  ( $15-22 \times 4-9\mu$ ).

On living leaves and inflorescence of *Capparis septaria* L. Hasanur (Coimbatore District) 16-8-1946, C. R. Venkataraman and Kallar (Coimbatore District) 9-10-1946, T. S. Ramakrishnan and K. Ramakrishnan.

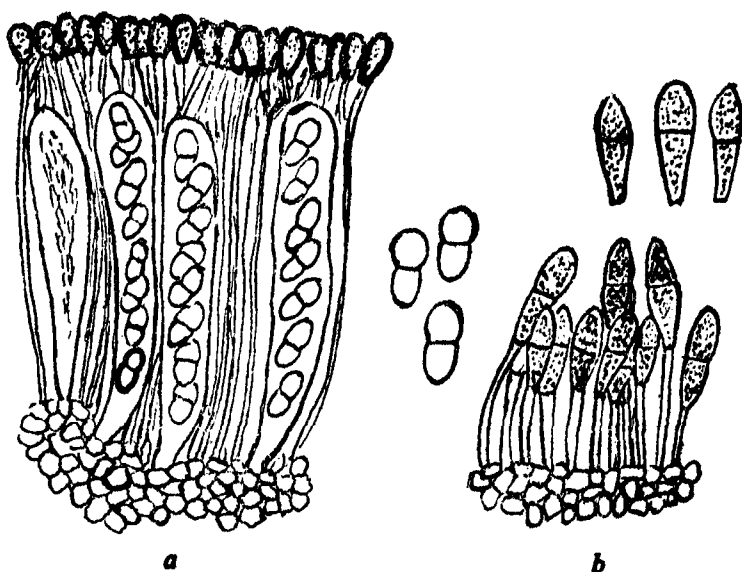
*Ascomata* amphigena, in incrassatis maculis foliorum, gregaria, turbinata, nigra, brevistipitata,  $400-700\mu$  diam. *asci* cylindrici vel clavati, hyalini, octosporiati,  $80 \times 12\mu$  ( $65-115 \times 10-17\mu$ ), *ascosporidia* duocellata, hyalina, oblique uniseriata, paraphysata  $13 \times 5.4\mu$  ( $10-19 \times 4.8-9.6\mu$ ), termini paraphyses ramifacti, rami juncti in fuscum epithecium; *pycnidia* in excrescentiis formata in inflorescentiis et fructibus, etiam ascomatibus mixta, cum irregulari tecto, quod erumpit variæ; *pycniosporidia* duo cellata, clavata levis olivacei colores.

\* 1947, Part III of this series was published in the *Proceedings of the Indian Academy of Sciences*, 26, No. 1.

Type specimens of all the fungi described have been deposited in the Herbarium of the Government Mycologist, Coimbatore, and the *Herb. Crypt. Ind. Orient.*, New Delhi.

In vivis foliis et inflorescentiis *Capparidis sepiuræ* L. Hasanur (Coimbatore District) 16-8-1946, C. R. Venkataraman et Kallar (Coimbatore District) 9-10-1946, T. S. Ramakrishnan et K. Ramakrishnan.

*Bulgariastrum caespitosum* Syd. has been recorded from the Philippines on *C. sepiaria*. But this fungus forms ascomata on the lower surface of the leaves. Further the pycnidia produce hyaline spores measuring  $26-45\mu$ . The fungus under study produces galls on the inflorescence also. The pycnidiospores are coloured and measure only  $15-22\mu$ , and thus it is different from the former. Dr. Mundkur to whom the type specimen was sent for comparison with *B. caespitosum*, available at the Herbarium Cryptogammæ Indiæ Orientalis, New Delhi, also found that the fungus under study was different. *B. africanum* Syd. has been recorded on *C. rudutis* from Natal. This fungus affects leaves only, producing ascomata, on both sides of the leaf. But it does not produce galls on the inflorescence. The pycnidial stage of this species has not been observed. For these reasons the present fungus is considered to be a new species and named *B. tumifaciens*.



TEXT-FIG. 1. *Bulgariastrum tumifaciens*.—(a) Section through a portion of apothecium showing asci and paraphyses; (b) Pycnidiospores ( $\times 600$ ).

(17) *Achroella plectroniæ* Ramakrishnan T. S. and K. sp. nov.

Stromata black, pulvinate, glomerulate, erumpent, amphigenous, developed in the middle of a circular, thickened, brown spot which forms a ring-like structure round the stroma, sparse or crowded, sessile attached by a

broad base to the leaf-tissue, subepidermal in origin; *perithecia* half immersed in the stroma, with a black outer wall, ovate to globose, ostiolate,  $240 \times 170 \mu$ , paraphysate, with fine filiform paraphyses; *asci* clavate, hyaline, with gelatinising wall, rounded at the ends and with a short narrow foot,  $82 \times 15 \mu$  ( $72-91 \times 14-19 \mu$ ); *ascospores* 8, fascicled, fusoid, tapering towards the ends, one-septate, light yellowish brown,  $44 \times 3 \mu$  ( $38-50 \times 2.5-3.5 \mu$ ).

*Pycnidia* of two kinds, one type resembling *Hemidothis*, developed in the stroma, associated with the perithecia or not, loculi sunk in the stroma, ostiolate; pycnidiospores long fusoid, curved, hyaline, one-celled,  $63 \times 3.5 \mu$  ( $40-76 \times 2-4.5 \mu$ ), produced on short hyaline stalks. The second type of pycnidium forms isolated, crowded, innate, erumpent, subepidermal, globose, ostiolate, dark structures; spores numerous, minute, spermatia-like; obviously these pycnidia function as spermogonia.

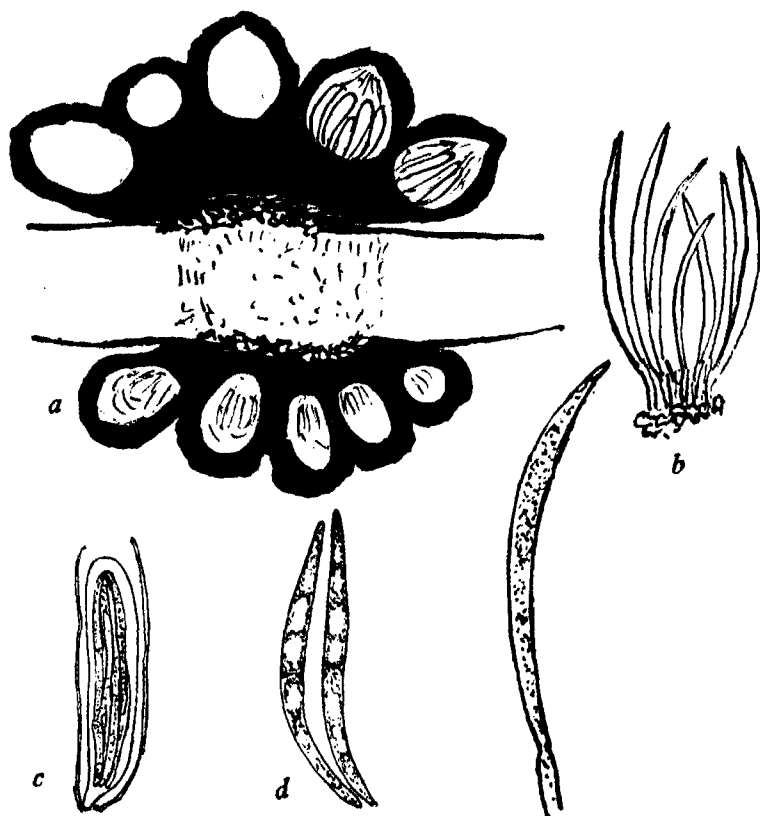
On living leaves of *Plectronia didyma* Kurz. Kallar (Coimbatore District), November 1946. T. S. Ramakrishnan.

Maculæ amphigenæ, orbiculares, incrassatæ; stromata nigra, glomerulata, amphigena; *perithecia* pars dimidia immersa, muris externi nigris, ovata vel globosa, ostiolata,  $240 \times 170 \mu$ , paraphysata, paraphyses filiformes; *asci* clavati, hyalini, murus *asci* gelatinus,  $82 \times 15 \mu$  ( $72-91 \times 14-19 \mu$ ); *ascosporidia* 8, fasciculata, fusoidea, angustata ad terminum, uniseptata, levis brunnei colores,  $44 \times 3 \mu$  ( $38-50 \times 14-19 \mu$ ).

Duo genera *pycnidium*, (1) sociata cum perithecia, immersa, ostiolata; *Pycnidiosporidia* longa, fusoidea, curvata, hyalina, unicellata,  $63 \times 3.5 \mu$  ( $40-76 \times 2-4.5 \mu$ ), pedicelli hyalini, brevis, (2) isolata amphigena, gregaria, innata, erumpentia, subepidermia, globosa, ostiolata, nigra; pycnidiosporidia numerosa, minuta, hyalina, baculoformia.

In vivis foliis *Plectronia didymæ* Kurz. Kallar (Coimbatore District), November 1946, T. S. Ramakrishnan.

The *Hemidothis*-like pycnidia are the most conspicuous. Perithecia are rare. Sydow (1916) in his description of *Hemidothis* has stated that it represents only the imperfect stage of a Dothidiaceous ascomycete, probably *Bagnisiopsis* or some related genera. In the present instance the perfect stage is represented by a fungus belonging to the Dothidiaceæ. Considering the structure of the stroma, asci and ascospores it has to be identified as *Achroella*. No allied fungus has been recorded on the present host. This fungus is considered to be a new species and named *A. plectroniæ*.



TEXT-FIG. 2. *Achroella plectronia*.—(a) Section through stromata (diagrammatic); (b) Pycnidiospores ( $\times 500-1000$ ); (c) ascus and paraphyses ( $\times 300$ ); (d) ascospores ( $\times 500$ ).

(18) *Hysterostoma pavetta* Ramakrishnan T. S. and K. sp. nov.

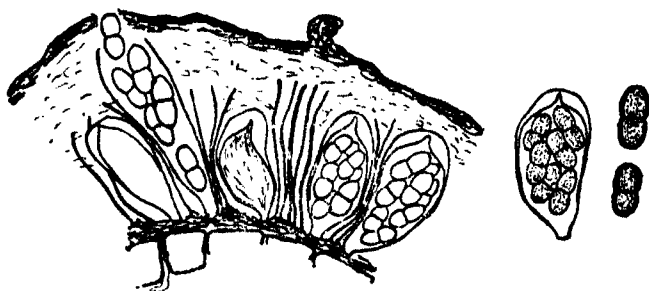
Spots yellowish, amphigenous, circular; stromata superficial, epiphyllous, dark; hypostroma subcuticular; haustoria sent into the epidermal and palisade cells, the tissue of the stroma gelatinises when moistened; asci stout, club-shaped, with rounded apex, and narrow base, wall-thickened and gelatinising near the top,  $50 \times 24 \mu$  ( $40-59 \times 19-33 \mu$ ), paraphysate, paraphyses fine, filiform; ascospores 8, smoky brown, 2-celled, oblong, constricted at the septum,  $21 \times 9 \mu$  ( $18-26 \times 7-11 \mu$ ).

On living leaves of *Pavetta indica* L. on the road from Kallar to Burliar (Coimbatore District), November 1946, T. S. Ramakrishnan.

Maculae amphigenae, flavae, orbiculares; stromata superficialia, epiphylla, fusca, hypostroma subcuticulare, haustoria penetrantia in cellae epidermes et paliformes; stroma gelatinum in aqua; asci crassi, clavati,

apice rotundato, basi attenuato, murus incrassatus et gelatinans ad summum,  $50 \times 24 \mu$  ( $40-59 \times 19-33 \mu$ ), paraphysata, paraphyses fragiles, filiformes; *ascosporidia* 8, fumide brunnei colores, duo cellata, oblonga, medio contracta,  $21 \times 9 \mu$  ( $18-26 \times 7-11 \mu$ ).

In vivis foliis *Pavetta indicæ* L. in via de Kallar ad Burliar (Coimbatore District), November 1946, T. S. Ramakrishnan.



TEXT-FIG. 3. *Hysterostoma pavettae*.—Stroma, ascus and ascospores ( $\times 300$ ).

This genus has not been recorded on this host so far from India or elsewhere. It is described as a new species. Hansford (1946) has described several species of this genus on various hosts but there is no mention of *Pavetta* among them.

(19) *Phæochorella artocarpi* Ramakrishnan T. S. and K. sp. nov.

Stromata amphigenous on the leaves, sometimes present on the petioles also, but more pronounced on the upper side along the midrib and main veins forming long raised areas; on the lower surface stromata less prominent and occupying corresponding positions or otherwise, black shiny, subcuticular, and made up of closely packed, dark brown, vertical hyphæ; location of the perithecia indicated by conical raised projections on the upper surface, loculi conical and much flattened,  $170 \times 86 \mu$ ; *asci* cylindric, clavate, 8-spored, hyaline,  $65 \times 16 \mu$  ( $52-89 \times 11-22 \mu$ ) paraphysate; *ascospores* light olive when young, and dark brown with age, elliptic, one-celled, uniseriate,  $14 \times 7 \mu$  ( $11-17 \times 4-9 \mu$ ); *pycnidia* found in the stromata on the lower surface of the leaf, ostiolate, of varying dimensions, immersed in the stromata or not, *pycnidiosporidia* oval to elliptic, one-celled, light olive,  $5.5 \times 4 \mu$  ( $4.5-7 \times 3-5 \mu$ ).

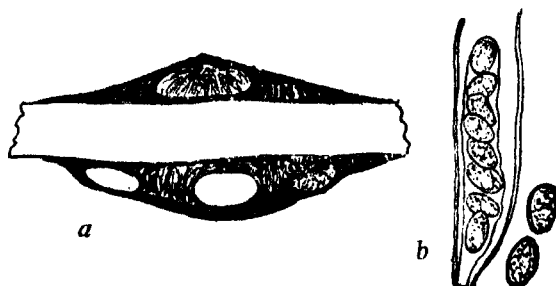
On living leaves of *Artocarpus Lakoocha* Roxb. Burliar (Nilgiris), November 1946, T. S. Ramakrishnan.

Stromata amphigena, mediam venam et prominentes venas sequentia, extanter epiphylla, nigra, micantia, sub-cuticularia, composita hyphis

verticalibus compactis; *perithecia* plurimum epiphylla, ostiolata, compressa,  $170 \times 86 \mu$ ; *asci* clavati, cylindrici, octosporiati, hyalini, paraphysati,  $65 \times 16 \mu$  ( $52-89 \times 11-22 \mu$ ); *ascosporidia* levis olivacei colores in juventute, fusci brunnei colores in maturitate, elliptica, unicellata, uniseriata,  $14 \times 7 \mu$  ( $11-17 \times 4-9 \mu$ ).

*Pycnidia* hypophylla, immersa in stromata; *pycnidiosporidia* ovalia vel elliptica, unicellata, levis olivacei colores,  $5.5 \times 4 \mu$  ( $4.5-7 \times 3-5 \mu$ ).

In vivis foliis *Artocarp* *Lakoocha* Roxb. Burliar (Nilgiris), November 1946, T. S. Ramakrishnan.



TEXT-FIG. 4. *Phaeochorella artocarpi*.—(a) Section through stromata (diagrammatic); (b) ascus and ascospores ( $\times 300$ ).

*Catacauma microcentum* (Berk. and Broome) Theiss. and Syd. has been recorded on *Ficus mysorensis*. The present fungus produces subcuticular stromata with closely packed vertically disposed hyphae, but it differs from *Catacauma* in having ascospores which are coloured when mature. It is therefore placed in the genus *Phaeochorella*. This genus has not been recorded on this host and the fungus is consequently named *P. artocarpi*. It is interesting to note that the pycnidial stage is confined to the lower surface of the leaf and the ascus stage to the upper surface. The tissues bordering the stromata sometimes develop a brown discolouration. Hyphal connection between the lower and upper surfaces of the leaf through the tissues is indistinct.

(20) *Puccinia luculenta* (Syd.) Ramakrishnan T. S. and K. comb. nov.

*Pycnia* not present; *aecia* sunk in the woody galls produced on both sides of the leaves; galls vary in size from 0.75–6 mm., almost spherical, dark brown, with varying numbers of *aecia* sunk in each gall; *aecia* present on both sides; each *aecium* has a definite peridium made up of one layer of thick-walled rectangular to polygonal warty cells; *aeciospores* globose or angular with a coarsely verrucose unequally thickened, hyaline wall,

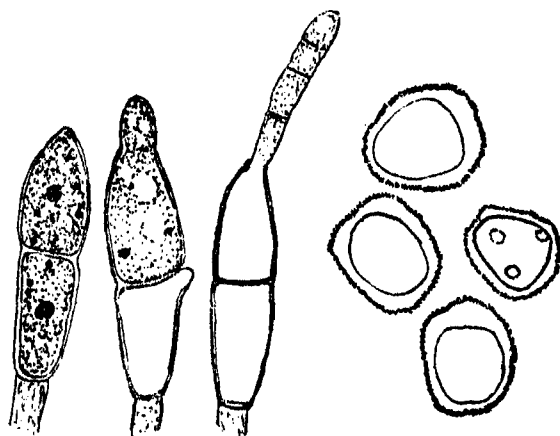
catenulate, germ pores two or more, clearly visible in younger spores.  $40 \times 35 \mu$  ( $34-48 \times 29-38 \mu$ ), orange coloured.

*Uredia* not observed; *telia* amphigenous, waxy, erumpent, cushiony, originating 2 to 3 layers below the epidermis which is burst through. The teliospores originate from a compact plectenchyma; each spore is stalked with a prominent, gelatinising, hyaline pedicel up to  $200 \mu$  in length; *teliospores* 2-celled, cylindrical, with tapering ends, slightly constricted at the septum, wall smooth, contents orange yellow in colour, with one germ pore in each cell; germ pore in the upper cell at the apex while the germ pore in the lower cell is just below the septum; spores measure  $77 \times 25 \mu$  ( $58-106 \times 19-34 \mu$ ), germinate immediately.

On living leaves of *Loranthus longiflorus* Desv. Attakatti (Anamalais), 10-4-1947, T. S. Ramakrishnan.

*Pycnia* ignota; *aecia* demersa in globosis amphigenis, lignieis excrescentiis; peridium unius strati cellularum polygonarum, crassomuratarum, echinularum; *aeciosporidia* globosa vel angularia, catenulata, exstanter verrucosa, murus inequaliter incrassatus, aurantiacei colores, porum germinationis unum vel plura; *uredia* absunt; *telia* amphigena, ceracea, erumpentia, pulvinata, subepidermia; *teliosporidia* stipitata, cum pedicellis exstanter gelatinantibus, hyalinis usque  $200 \mu$  longa, teliosporidia duocellata, cylindrica, angustatis terminis, medio leviter constricta, episporio levi, contenta aurantiacei flavi colores, quaque loculo porum germinationis unum gerente  $77 \times 25 \mu$  ( $58-106 \times 19-34 \mu$ ) germinantia *in situ*.

In vivis foliis *Loranthi longiflori* Desv. Attakatti (Anamalais), 10-4-1947, T. S. Ramakrishnan.



TEXT-FIG. 5. *Puccinia luculenta*—teliospores and aeciospores ( $\times 300$ ).



Several rusts have been observed on *Loranthus*. From Mysore Butler and Bisby (1931) have recorded *Aecidium luculentum* Syd. of which only æcia were observed on leaves and stem of *L. longiflorus*. *Aecidium Schimperi* Bacc. has been observed on *L. Schimperi* from tropical Africa. On *Loranthus crassipes* and *Loranthus* sp., *Uromyces socius* Arth. and Holw. has been recorded from Central America. McAlpine (1906) has described *P. loranthicola* McAlp. on *L. celastroides*, from Australia. *P. loranthi* Speg. on *L. cordatus* and *P. macrocarya* Racib. on *L. pentandra* have also been reported. Cummins (1941) has recorded *P. heroica* Cum. and *P. macrocarya* on *Loranthus* spp. from New Guinea. *P. loranthicola* produces æcia, uredia and telia. But æcia are found on separate leaves. The teliospores are intermixed with the urediospores. In these it differs from the rust under study, which does not form uredia, and the æcia and telia are formed on the same leaf. *P. loranthi* forms only telia and the spores are smaller than those of the present rust. *P. macrocarya* and *P. heroica* form pycnia. The teliospores are yellowish (or nearly hyaline in *P. macrocarya*) and the pedicels are concolorous. Spots are formed round the hypophyllous telia of *Puccinia heroica*. In the rust under study pycnia have not been found. The telia are amphigenous and no spot is visible surrounding the telium. The stalks of teliospores are hyaline while the teliospores are orange yellow in colour. Further, the stalks gelatinise readily when moistened. In these characters it differs from both the above species. A comparative statement of these rusts is given below.

	Aeciospores	Teliospores	Remarks
<i>P. heroica</i> ..	35-48 × 45-65 μ	60-26 × 18-23 μ	Pycnia present, æciospore with apical thickening, telia hypophyllous, teliospores yellowish, with concolorous stalks.
<i>P. macrocarya</i> ..	33-39 × 39-46 μ	72-89 × 23-30 μ	Pycnia prominent; æcia mostly hypophyllous, telia amphigenous, teliospores yellowish with concolorous stalks.
<i>P. luculenta</i> ..	34-48 × 29-38 μ	58-106 × 19-34 μ	Pycnia absent, æciospore with unequally thickened wall with 2 or more germ pores, telia amphigenous, teliospores, orange yellow, pedicel hyaline and gelatinising

Butler and Bisby (1931) have described *A. luculentum* on *Loranthus longiflorus* from Mysore. The æcia of the rust under study resemble those of *A. luculentum*. Since the perfect stage has now been noticed on the same host mixed with the æcia a new combination *P. luculenta* is proposed.

The gelatinisation of the stalks of the teliospores is very prominent and resembles what occurs in *Gymnosporangium*. Germ pores are visible in the young aeciospores. These characters have not been noticed in the other species of *Puccinia* recorded on this host.

(21) *Hapalophragmium anamalaiensis* Ramakrishnan T. S. and K. sp. nov.

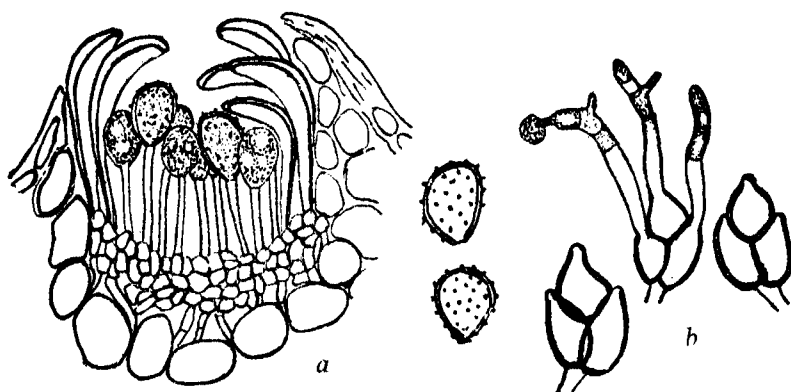
*Pycnia* and *aecia* not known; *uredia* hypophyllous, minute, scattered, light brown, erumpent, subepidermal in origin; *urediospores* stipitate, oval to elliptic, brown to orange, echinulate,  $34 \times 23 \mu$  ( $24-38 \times 19-27 \mu$ ), paraphysate, paraphyses incurved light brown, thickened on the outer side at the upper portion and not on the inner side, forming a protective covering for the sorus; *telia* hypophyllous, darker in colour than the uredia, minute, erumpent, scattered, subepidermal, in origin, paraphysate, paraphyses like those of the uredia; *teliospores* stipitate, stalk hyaline; rhomboidal in shape, three-celled, with two lower cells and one upper; stalk attached at the base of the two lower cells at the indentation; the two lower cells are elongated and are in contact for half their lengths, the top cell is wedged in between these, and rhomboidal in shape; wall smooth and thin, light brown in colour, with one germ pore at the tip of each cell; spores measure  $43 \times 28 \mu$  ( $35-58 \times 17-34 \mu$ ), germinate immediately without a period of rest, producing one hyaline promycelium from each cell; promycelium coming out through the germ pore, four-celled with one basidiospore from each cell.

On living leaves of *Derris eulata* Bedd. Anamalais, 10-4-1947, T. S. Ramakrishnan.

*Pycnia* et *aecia* ignota; *uredia* hypophylla, minuta, levis brunnei colores, subepidermia; *urediosporidia* stipitata, ovata vel elliptica, brunnei vel aurantiacei colores, echinulata  $34 \times 23 \mu$  ( $24-38 \times 19-27 \mu$ ), paraphysata; paraphyses incurvatæ, levis brunnei colores, externæ incrassatæ; *telia* hypophylla, fusciora quam uredia, minuta, erumpentia, subepidermia, paraphysata ut urediis; *teliosporidia* stipitata, rhomboidea, 3-cellata, 2 infra et una supra, unum porum germinationis in quaque cella in apice, brunnei colores,  $43 \times 28 \mu$  ( $35-58 \times 17-34 \mu$ ), germinantia sine quiete.

In vivis foliis *Derridis eulatae* Bedd. Anamalais, 10-4-1947, T. S. Ramakrishnan.

*H. derridis* Syd., *H. setulosum* (Pat.) Syd., and *Triactella pulchra* (Rac.) Syd., have been recorded on *Derris uliginosa*, *Derris* sp., and *D. elliptica*, respectively. Sydow and Petrak (1931) are of opinion that *T. pulchra* has to be renamed *H. pulchrum* and that the genus *Triactella* which was founded



TEXT-FIG. 6. *Haplophragmium anamalaiensis*. (a) uredium and urediospores ( $\times 300$ ); (b) teliospores ( $\times 300$ ).

on *T. pulchra* has to be abandoned. The rust under study is a *Haplophragmium*, but it differs from the previously recorded species in several respects. *H. setulosum* (Miles and Traverse, 1904) and *H. pulchrum* (Sydow and Petrak, 1931) have warts near the germ pores of the teliospores, whereas in the rust under study the teliospores are perfectly smooth. The sori of *H. derridis* are amphigenous and paraphyses have not been recorded. In the rust under study, however, the sori are hypophyllous and numerous characteristic paraphyses are present. For these reasons this rust is found to be different and is, therefore, named *H. anamalaiensis*.

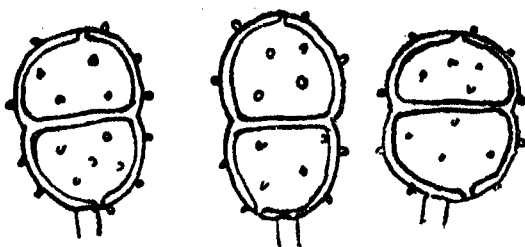
(22) *Puccinia vernoniae-monosis* Ramakrishnan T. S. and K. sp. nov.

*Pycnia*, *æcia* and *uredia* absent; *telia* hypophyllous, sparse, erumpent, sub-epidermal, cushion-shaped, with the leaf thickened at the affected region, dark brown in colour; *teliospores* stipitate, with a short hyaline stalk, 2-celled, elliptic to subglobose, rounded at the apex and base, thick-walled, prominently echinulate, with sparse hyaline echinulations, reddish brown in colour,  $35 \times 27 \mu$  ( $29-43 \times 24-34 \mu$ ), germ pore one in each cell.

On living leaves of *Vernonia monosis* C. B. Clarke, Valparai (Anamalais), 10-4-1947, T. S. Ramakrishnan.

*Pycnia æcia et uredia non cognita*; *telia* hypophylla, sparsa, erumpentia, fusca; *teliosporidia* stipitata, duocellata, elliptica vel sub-globosa, exstanter sparse echinulata, rubrei brunnei colores,  $35 \times 27 \mu$  ( $29-43 \times 24-34 \mu$ ).

In vivis foliis *Vernonia monosis* C. B. Clarke, Valparai (Anamalais), 10-4-1947, T. S. Ramakrishnan.



TEXT-FIG. 7. *Puccinia vernoniae-monospora*.—Teliospores ( $\times 600$ ).

The host plant is arboreal in habit and represents one of the three tree species of *Vernonia* in South India. Jackson (1918, 1932) has given a list of *Puccinia* occurring on species of *Vernonia*. Over fifty species of *Puccinia* have been included in this list. These have been classified into two groups, having smooth or rough-walled teliospores. The rust under study is a microform having only the III stage. It has teliospores with prominent sparsely placed echinulations and is different from other recorded species. Further no *Puccinia* has been recorded on the present host. Therefore, the rust is considered a new species and named *Puccinia vernoniae-monospora*.

(23) *Aecidium terminaliae* Ramakrishnan T. S. and K. sp. nov.

Spots amphigenous, sparse, yellow to reddish brown, with a distinct more or less spherical, woody gall in the centre; the gall projects out on both sides of the leaf; sometimes the galls are formed along the margins of the leaves also. *Pycnia* amphigenous, subcuticular hemispherical; *pycniospores* hyaline, spindle-shaped; *aecia* sunk in the woody galls, varying numbers of cupulate hard-rimmed *aecia* present in each gall, *aecia* measure  $670 \times 360 \mu$ ; peridium ephemeral, made up of polygonal smooth-walled cells; *aeciospores* catenulate, polyhedral, with thick walls, one end sometimes thicker than the other, densely and prominently verrucose, measuring  $42 \times 24 \mu$  ( $29-49 \times 19-29 \mu$ ), deep reddish brown.

On living leaves of *Terminalia bellerica* Roxb., Valparai, 10-4-47, T. S. Ramakrishnan.

Maculae sparsae, amphigenae, brunnei colores, cum distincto, spherico, ligneo, excrescente projiciente ambabus lataribus; *pycnia* amphigena, subcuticularia, hemispherica; *pycniosporidia* hyalina, fusiformia; *aecia* immersa, cupulata,  $670 \times 360 \mu$ , peridii evanescentis, cellulis polygonis, pariete levi; *aeciosporidia* catenulata, polyhedra, interdum inequaliter incrassata, exstanter et dense verrucosa,  $42 \times 24 \mu$  ( $29-49 \times 19-29 \mu$ ), dense rubrei-brunnei colores.

In vivis foliis *Terminalia bellerica* Roxb., Valparai (Anamalais), 10-4-1947, T. S. Ramakrishnan.



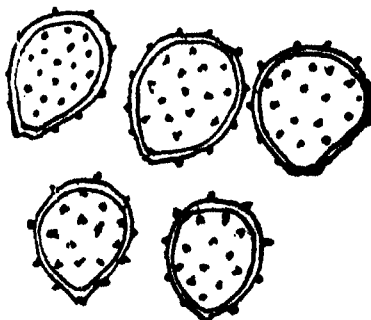
TEXT-FIG. 8. *Aecidium terminaliae* aeciospores ( $\times 300$ ).

The only rust recorded on this host genus is *Uredo terminaliae* P. Henn. on *T. argentea* and *T. hyalobotes*. In the fungus now collected only the aecial stage is observed and it is therefore for the present included under the form genus *Aecidium* and is named *A. terminaliae*.

(24) *Uredo amomi* Petch

Spots small, yellowish brown, isolated amphigenous; *uredia* hypophyllous, minute, pulverulent, one or more in a spot, whitish, subepidermal; *urediospores* ovate, globose, strongly spinulose, stipitate, hyaline to light yellow,  $28 \times 24 \mu$  ( $24-38 \times 19-29 \mu$ ).

On living leaves of *Amomum* sp., Valparai (Anamalais), 10-4-1947, T. S. Ramakrishnan.



TEXT-FIG. 9. *Uredo amomi*.—Urediospores ( $\times 600$ ).

A similar rust was prevalent on the leaves of *Elettaria cardamomum* on the Anamalais during the same period. Yellowish brown spots are visible on the upper surface. Minute uredia develop in clusters on the lower surface of the spots. The uredia are whitish to pale brown in colour. Urediospores are oval to globose, strongly spinulose, hyaline to light yellow and measure  $22-32 \times 20-28 \mu$ .

A comparison of the above two rusts exhibited complete agreement in all characters.

*Phakopsora elettariae* (Rac.) Cumm. has been recorded on *Amomum* sp. from New Guinea. Cummins (1941) had concluded that the description of the telia of *Schroeteriaster elettariae* Rac. indicated that it was really a *Phakopsora*. *S. elettariae* was recorded on *Elettaria cardamomum* by Raciborski 1900. *Uredo amomi* Petch has been described from Ceylon (Petch, 1911-14) on *Amomum involucratum*. A comparative statement of *P. elettariae*, *U. amomi* and the two present rusts is given below.

	Urediospore dimensions	Remarks
<i>P. elettariae</i> ..	24-30 × 15-20 $\mu$	Orange yellow, finely verruculose
<i>U. amomi</i> ..	25-38 × 10-23 $\mu$	Strongly spinulose, hyaline
Rust on <i>amomum</i> ..	24-38 × 10-29 $\mu$	Hyaline to light yellow, strongly spinulose
Rust on <i>Cardamomum</i> ..	23-32 × 20-28 $\mu$	Hyaline to light yellow, strongly spinulose

It is evident from the above statement that the present rusts are identical with *U. amomi* in spore size, and shape and in the nature of the wall. Some of the spores in the present rusts have a light yellowish colour, which however cannot be taken as a valid differentiating character. It is very probable that all the three rusts represent only the uredial stage of *P. elettariae*. But since only the uredia have been seen they are tentatively placed in *U. amomi*.

#### (25) *Uredo colebrookiana* Barclay.

On leaves of *Colebrookia oppositifolia* Sm., Valparai (Anamalais), 10-4-1947, T. S. Ramakrishnan.

This rust has not been observed in South India before.

#### ACKNOWLEDGEMENT

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1



2



3

*Bulgariastrum tumefaciens*

1. Leaves and inflorescence of *Capparis sepiaria*, showing galls
2. Apothecia (  $\times 10$  )
3. Section of Apothecium (a) and Pycnidium (b) (  $\times 100$  )





# AMIDE SYNTHESIS IN PLANTS

## IV. Aspartase in Germinating Seedlings

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Received December 16, 1947

SINCE the discovery by Quastel and Woolf (1926) of the enzyme aspartase which converts aspartic acid into fumaric acid and ammonia its activity in several micro-organisms has been studied. Virtanen and Tarnanen (1932) prepared the enzyme from *Bacterium fluorescens liquifaciens* while Gale (1938) was able to show its presence in *B. coli*. According to Hæhn and Leopold (1937) autolysates of bottom yeast are also capable of converting aspartic acid to fumaric acid and ammonia. The only observation on the occurrence of this enzyme in plants was made by Virtanen and Tarnanen (1932) who found it to be present in pea seedlings and leaves of young red clover. The significance of aspartase in the reactions involved in amide synthesis in plants is obvious. The formation of aspartic acid from succinic acid can take place by one of two routes—either by the amination of oxalacetic acid formed from succinic acid *via* fumaric and malic acids or by the amination of fumaric acid itself by the synthetic action of the enzyme aspartase. Investigations were therefore carried out to ascertain the presence of this enzyme in seedlings of *Phaseolus mungo* and *Cicer arietinum*, previously studied in this laboratory from the point of view of amide synthesis, as well as in *Pisum sativum* which was investigated by Virtanen and Tarnanen.

### EXPERIMENTAL

In germinating the seeds special precautions were taken to sterility in view of the strong aspartase activity of bacteria. The seeds were first soaked in 95% alcohol for three minutes, washed in water and then placed in 0.2% mercuric chloride solution for a further period of three minutes. They were then washed thoroughly with water, soaked in water for twenty-four hours and germinated on sterile sand in large vessels. For determination of enzyme activity a uniform suspension of the finely macerated seedlings was used; after removal of the testa the seeds or seedlings

were washed with water, dried lightly on filter-paper and then ground thoroughly in a cooled granite mortar. The ground brei after being dispersed in sufficient water to make 10 ml. of suspension for 1 gm. of fresh material was allowed to autolyse under toluene for twenty-four hours at 37° before use. The *L*-aspartic acid which was used as substrate was prepared by hydrolysis of asparagine with 3N hydrochloric acid for three hours. The solution was neutralised with ammonia and the aspartic acid precipitated was washed free from chloride with ice-water. It was recrystallised from hot water and its purity established by determination of the nitrogen content.

The experimental solutions for studying enzyme activity were made up as follows, the aspartic acid used being first neutralised to sodium aspartate before being added to the enzyme.

Enzyme suspension	..	..	..	20 ml.
Aspartic acid	..	..	..	0.4 gm.
M/15 Phosphate buffer (pH 7)	..	..	..	10 ml.
Total volume	..	..	..	50 ml.

Toluene was used as antiseptic and the solutions kept at 37° during the experimental period. In several experiments sodium fluoride was added to a concentration of 0.5% to exclude the possibility of bacterial action. A control was run without the addition of aspartate.

Enzyme activity was determined by periodical estimations of ammonia. 10 ml. of the experimental solution was introduced into a 50 ml. centrifuge tube containing 2.5 ml. of 20% trichloroacetic acid. The solution was then centrifuged for five minutes and the centrifugate filtered through paper into a 25 ml. standard flask. The residue in the centrifuge tube was washed once with 10 ml. of 2.5% trichloroacetic acid and the washings after centrifuging and filtration combined with the first filtrate. The solution was now brought to pH 7 and the ammonia determined according to the method of Pucher *et al.* (1935) by distilling *in vacuo* at 40° in Parnas and Klisiecki apparatus. The ammonia liberated was absorbed in sulphuric acid, nesslerised and determined colorimetrically. A similar estimation was simultaneously made on an aliquot of the control solution containing no aspartic acid.

## RESULTS

In the following tables ammonia is expressed in mg. per 10 ml. of the reaction mixture.

TABLE I

## Phaseolus mungo, 2-day old seedlings

(a) without sodium fluoride

(b) with sodium fluoride

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	a. 0.102	0.11	..	..	0.008
	b. 0.102	0.102	..	..	..
48	a. 0.141	0.216	0.039	0.106	0.067
	b. 0.141	0.196	0.039	0.094	0.056
96	a. 0.163	0.253	0.061	0.143	0.082
	b. 0.153	0.230	0.061	0.130	0.079
168	a. 0.163	0.198	0.061	0.288	0.227
	b. 0.153	0.358	0.051	0.256	0.205

TABLE II

## Phaseolus mungo, 4 day old seedlings

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	a. 0.238	0.240	..	..	0.002
	b. 0.240	0.240	..	..	..
48	a. 0.422	0.407	0.184	0.227	0.043
	b. 0.427	0.407	0.187	0.227	0.040
96	a. 0.495	0.565	0.257	0.325	0.068
	b. 0.498	0.563	0.258	0.323	0.065
168	a. 0.495	0.616	0.257	0.376	0.119
	b. 0.498	0.600	0.258	0.360	0.102

TABLE III

## Phaseolus mungo, 6-day old seedlings

Time (Hours)	Ammonia gm.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	0.232	0.232	..	..	..
48	..	..	Not determined		..
96	0.512	0.560	0.280	0.318	0.038
168	0.512	0.584	0.280	0.332	0.072

TABLE IV

*Cicer arietinum*, 3-day old seedlings

(a) without sodium fluoride

(b) with sodium fluoride

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	..	a. 0.262 b. 0.260	0.266 0.260	.. ..	0.004 ..
96	..	a. 0.380 b. 0.378	0.436 0.450	0.118 0.118	0.072 0.072
168	..	a. 0.380 b. 0.378	0.558 0.552	0.118 0.118	0.174 0.174

TABLE V

*Cicer arietinum*, 5-day old seedlings

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	..	0.389	0.390	..	0.001
48	..	0.451	0.475	0.062	0.023
96	..	0.575	0.628	0.146	0.092
168	..	0.535	0.793	0.146	0.257

TABLE VI

*Cicer arietinum*, 7-day old seedlings

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	..	0.420	0.420	..	..
48	..	0.496	0.515	0.076	0.019
96	..	0.612	0.696	0.192	0.084
168	..	0.615	0.811	0.195	0.196

TABLE VII

*Cicer arietinum*, 11-day old seedlings

Time (Hours)	Ammonia mg.				
	Contr. I	Experimental	Increase		Difference
			Control	Experimental	
0	0.463	0.463	..	..	..
48	0.510	0.520	0.047	0.057	0.010
96	0.542	0.587	0.079	0.124	0.045
168	0.542	0.642	0.079	0.179	0.103

TABLE VIII

*Pisum sativum*, 3-day old seedlings

(a) without sodium fluoride

(b) with sodium fluoride

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	a. 0.175	0.175	..	..	..
	b. 0.175	0.175	..	..	..
48	a. 0.212	0.203	0.037	0.088	0.051
	b. 0.209	0.260	0.054	0.085	0.051
96	a. 0.297	0.407	0.122	0.232	0.110
	b. 0.297	0.390	0.122	0.224	0.102
168	a. 0.300	0.492	0.125	0.317	0.192
	b. 0.300	0.492	0.125	0.317	0.192

TABLE IX

*Pisum sativum*, 5-day old seedlings

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0	0.252	0.255	..	..	0.003
48	0.376	0.451	0.124	0.196	0.072
96	0.499	0.648	0.247	0.293	0.146
168	0.500	0.720	0.248	0.465	0.217

TABLE X  
*Pisum sativum*, 7-day old seedlings

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	0.387	0.387	..	..	..
48 ..	0.514	0.594	0.127	0.197	0.070
96 ..	0.605	0.770	0.218	0.283	0.165
168 ..	0.605	0.897	0.218	0.510	0.292

TABLE XI  
*Pisum sativum*, 11-day old seedlings

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	0.465	0.465	..	..	..
48 ..	0.682	0.738	0.217	0.273	0.056
96 ..	0.736	0.848	0.271	0.383	0.112
168 ..	0.740	0.935	0.275	0.470	0.195

### DISCUSSION

It is clear from the results obtained that aspartase is present in the three seedlings examined. The possibility that the decomposition of aspartic acid in these experiments was brought about by bacterial contamination is definitely ruled out as the activity was unaffected by the presence of fluoride and toluene.

The observed activity was, however, not very strong being of the same order as was observed by Virtanen and Tarnanen; the amount of aspartic acid decomposed at the time of maximum enzyme activity was between 4 and 5 mg. per gm. of seedling material during the experimental period of 168 hours. Further the activity of the enzyme diminished with the growth of the seedlings being maximum in *Phaseolus mungo* on the second day, in *Cicer arietinum* on the fifth day and in *Pisum sativum* on the seventh day after germination. The explanation for the low concentration of aspartase may be that the function of the enzyme in plants is a synthetic one and that its activity is therefore dependent upon the integrity of the cell structure; or

alternatively that the main path of aspartic acid synthesis in plants is *via* oxalacetic acid—the importance of which in carbohydrate metabolism is indicated by several lines of evidence—and that aspartase plays only a secondary role in the synthesis of aspartic acid from succinic acid.

#### SUMMARY

The presence of the enzyme aspartase which converts aspartic acid into fumaric acid and ammonia has been demonstrated in seedlings of *Phaseolus mungo*, *Cicer arietinum* and *Pisum sativum*. Its significance in aspartic acid synthesis in plants is briefly discussed.

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# STUDIES IN THE GENUS PHYTOPHTHORA—II

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IN the previous article Thomas *et al.* (1947) discussed the behaviour of different isolates of *Phytophthora palmivora* in paired cultures and based on the results of their studies, all the isolates studied were brought under the same species, viz., *P. palmivora* Butl. though they were being previously referred to three or more different species. Further investigations were continued with these and some more isolates belonging to *P. parasitica* and *P. colocasiae*. The results of these investigations are described in this paper.

## MATERIALS AND METHODS

As mentioned in the earlier communication (Thomas *et al.*, 1947) the isolates of *Phytophthora* already available in stock collection in the section were utilised. New isolates were brought into culture from infected castor and agave leaves when infection by the fungus occurred in nature on the Central Agricultural Research Station, Coimbatore. Isolates from bread-fruit were obtained from diseased fruits got down from South Kanara. Authentic type cultures of *P. parasitica* and *P. colocasiae* were received from the Indian Agricultural Research Institute, New Delhi, through the courtesy of Mr. Dastur. Dr. Asthana was kind enough to send us from Nagpur the culture of *P. parasitica* var. *piperina*. The paired cultures were grown on oat agar in petri-dishes or test-tubes in the months of October to January when the laboratory temperature was in the neighbourhood of 26° C.

### 1. Isolate from castor (*Ricinus communis*)

Castor plants growing in the experimental plots of the Oilseeds Specialist at Coimbatore were found infected during the north-east monsoon in October 1946, by a leaf-blight caused by *Phytophthora*. The fungus was isolated and brought into pure culture from single hyphal tips. A white luxuriant aerial growth developed on oat agar. Numerous ob-pyriform sporangia and intercalary hyaline to yellowish brown chlamydospores were formed. But sexual bodies did not develop even after four months.

This isolate was grown in paired cultures with other isolates and oospores were formed in the course of one week as shown in Table I.

TABLE I

*Statement showing the result of growing this isolate in paired cultures*

Paired with isolate from		Result		
Arecanut	<i>P. palmivora</i> plus strain	..	Oospores formed in 6 days	
Clerodendron	do do	..	do do	5 do
Agave	do minus strain	..	do do	5 do
Hevea	do do	..	do do	5 do
<i>P. parasitica</i> type culture		..	do do	4 do
<i>P. colocasiae</i>		..	do do	5 do

Oospores were formed with both the plus and minus strains of *P. palmivora* and also with *P. parasitica* and *P. colocasiae*. In single strain cultures however oospores are not developed either by this isolate or by the others with which it was paired. *P. parasitica* has been recorded on castor and the isolate under study will normally be identified as *P. parasitica* from the sporangial and chlamydospore characters though it does not form oospores in single strain cultures. But the ease with which it pairs with the plus and minus strains of *P. palmivora* raises some important doubts about the taxonomic relationship of the former species. This is discussed later in detail.

## 2. Isolate from *Agave wightii*

The leaf-blight on this host caused by *Phytophthora* was prevalent at Coimbatore in the months of November and December 1946. The isolate was brought into pure culture by tissue culture which was further purified by transferring single hyphal tips. On oat agar a luxuriant white aerial growth which filled the tube was formed. Profuse formation of sporangia and chlamydospores took place. No sexual bodies developed even after four months.

Paired cultures were grown using this isolate as one of the strains and one or another isolate of known performance. The results are given in Table II.

The behaviour of this isolate is identical with that of the old isolate from the same host already available in the section (Thomas *et al.*, 1947) in the formation of oospores with strains of *P. palmivora*. But the old isolate is reported (Marudarajan, 1941) to have produced oospores in single strain cultures for some time after isolation though this capacity was lost after two years' continuous culturing on agar media. The new isolate however does not form oospores in single strain cultures even after three months. The isolate readily combines with *P. parasitica* and *P. colocasiae* also and forms oospores in paired cultures with these species.

TABLE II

Statement showing the results of growing the isolate from  
*Agave wightii* in paired culture

Paired with isolate from	Results
Clerodendron <i>P. palmivora</i> plus ..	Oospores formed in 5 days
Arecanut do do ..	do do 5 do
Hevea do minus ..	No oospores formed
Spondias do do ..	do
Castor <i>P. parasitica</i> ..	Oospores formed in 3 days
<i>P. parasitica</i> type culture ..	do do 5 do
<i>P. parasitica</i> var <i>piperina</i> ..	do do 5 do
<i>P. colocasia</i> type culture ..	do do 5 do

### 3. Isolate from Breadfruit (*Artocarpus incisa*)

The performance of two earlier isolates from this host have been described in an earlier communication (Thomas *et al.*, 1947). This isolate was obtained from infected fruits of *Artocarpus incisa* brought from South Kanara. Its growth on oat agar was less profuse than the isolates from castor and agave. Ob-pyriform sporangia which are usually terminal and hyaline, and yellowish thick-walled spherical or subglobose intercalary chlamydospores are formed in large numbers. Sexual reproduction is not evident for the first ten days of growth. But numerous oogonia and oospores are formed in 15 days in single strain cultures. The culture was isolated from single hyphal tips and therefore this isolate is found to be homothallic and fertile. The oogonium is hyaline or light yellow and persistent. The antheridium is amphigynous. The oospore is thick walled and yellowish brown. The relative measurements of oogonia and oospores were 28.6 (20–31)  $\mu$  and 27.5 (18–29)  $\mu$  respectively. These are not far different from the average of the measurements obtained by Marudarajan (Thomas, 1941) for the sexual bodies of an isolate from breadfruit, *viz.*, oogonia 30.5  $\mu$  (19.3–38.5)  $\mu$  and oospores 28.3 (17.5–35)  $\mu$ .

This isolate from breadfruit was also grown in paired cultures with other isolates whose sexual behaviour was already known and the influence on the rapidity of oospore formation was observed.

From the results below it is found that the isolate from breadfruit forms oospores in single strain cultures in 11 days. But when paired with the plus strain of *P. palmivora* or with *P. parasitica* or *P. colocasia* sexual bodies begin to develop within thirty-six hours to 3 days after inoculation. On the other hand, when it is paired with the minus strains of *P. palmivora* oospore formation is visible only after ten days. In these the oospores

TABLE III

Statement showing the effect of pairing the isolate from breadfruit with other isolates of *Phytophthora*

Paired with isolate from				Results		
Jack	<i>P. palmivora</i>	plus strain	..	Oospores formed in	48	hours
Betelvine	do	do	..	do	do	48 do
Citrus	do	do	..	do	do	48 do
Colocasia	do	do	..	do	do	48 do
Ar. ca	do	do	..	do	do	48 do
Clerodendron	do	do	..	do	do	36 do
Spondias	do	minus strain	..	No oospores upto	10	days
Iieva	do	do	..	do	do	
<i>P. parasitica</i>		type culture	..	Oospores formed in	3	do
<i>P. colocasiae</i>		do	..	do	do	3 do
Isolate from breadfruit (single strain)			..	do	do	11 do

might have been formed by the breadfruit isolate itself and not by the pairing of the two strains. Thomas *et al.* (1947) have found that the first isolate of *P. palmivora* from breadfruit behaved as a minus strain. It formed oospores in single strain cultures when first isolated by Marudarajan (Thomas, 1941) but this capacity had been lost in later years, after having been grown on agar media for over four years. Thus a homothallic fertile strain has apparently changed later on into a minus strain. The present isolate also gives an indication of its probable future change. At present it is homothallic and fertile but it pairs easily with plus strains of *P. palmivora* or with homothallic non-fertile strains of *P. parasitica* or *P. colocasiae* while there is no evidence of combination with the minus strains, thus exhibiting a tendency towards the development of the 'minus' character.

Gadd (1927) obtained an isolate from breadfruit which behaved as a member of his 'rubber' group (which is equivalent to the minus strain of *P. palmivora*). Apparently breadfruit is parasitized by both homothallic (fertile) and minus strains of *P. palmivora* in different localities. But eventually even the homothallic strain changes into the minus strain when grown on agar media for a length of time.

#### 4. *P. parasitica* Dast. var. *piperina* Dast. on Piper betle

A type culture of this isolate was kindly supplied by Dr. Asthana, the Mycologist to the Government of Central Provinces and Berar, Nagpur. It was found to grow luxuriantly on oat agar forming a white dense aerial growth. Dastur (1935) had described this as a new variety of *P. parasitica*. His isolates were producing numerous oospores on agar media. But the culture received from Nagpur formed sporangia and chlamydospores but

not sexual bodies. It was however grown in paired cultures with other isolates of known reaction and the results are given below.

TABLE IV

*Statement showing the formation of oospores in paired cultures*

Paired with the isolate from			Results
Aecanot (Tyagli)	<i>P. palmivora</i> minus strain	..	Many oospores in 8 days
Coconut	do	..	Less number in 6 do (Oogonia deep coloured thickened)
Hevea	do	..	Numerous oo-pores in 4 days
Jatropha	do	..	do do 5 do
Agave	do	..	do do 4 do
Spondias	do	..	Less number do 4 do
Tomato	do plus strain	..	do do 8 do
Areca	do	..	do do 8 do
<i>P. parasitica</i>		..	Numerous oospores 4 do
<i>P. colocasiae</i>		..	do do 7 do

This isolate combines with both the plus and minus strains of *P. palmivora*, with *P. parasitica* and *P. colocasiae*. In this respect it is akin to the isolate from castor. The intensity of oospore formation varies in the different combinations. Oospores are formed in greater abundance with isolates recently brought into culture than with older ones.

Dastur (1935) raised this isolate to a new variety of *P. parasitica*, the main reason being the larger sizes of the oogonium and the oospore compared to those of *P. parasitica* though he acknowledges on the same page that "the size of the oogonium is not constant and is influenced by the medium". In later isolations of *P. parasitica* he had found bigger oogonia and oospores. In authentic cultures of *P. parasitica*, Tucker (1931) has found that oospores are very variable in size and exhibit a range in diameter from 12 to 35  $\mu$ . In the light of the above observations it becomes evident that this isolate is not different from the type of *P. parasitica* and there is not enough justification to classify it as a new variety.

The sexual bodies formed in some of the paired cultures were measured and the measurements are given below together with the sizes recorded by Dastur.

These measurements fall within the range of those recorded from paired cultures of *P. palmivora* strains (Thomas *et al.*, 1947).

TABLE V

Statement showing the size of sexual bodies in paired cultures

Particulars	Oogonia		Oospores	
	Mean $\mu$	Range $\mu$	Mean $\mu$	Range $\mu$
<i>P. parasitica</i> var <i>piperina</i> Dastur's measurements	38.4	20.4-40.8	26.1	17.8-33.1
do + <i>P. palmivora</i> (Tyagli)		24 -32		22 -27
do + do Tomato		24 -33		21 -30
do + do Coconut	31.5	26 -43	26	20 -31
do + <i>P. parasitica</i> ..	28.0	21 -34	23	16 -25
do + <i>P. colocasiae</i> ..	29.1	23 -33	22.5	18 -26

5. *P. colocasiae* Rac.

A type culture of this fungus was obtained from the Indian Agricultural Research Institute, New Delhi, through the courtesy of the Head of the Division of Mycology. Its growth on oat-agar is not so luxuriant as that of the isolates from betelvine or arecanut. The cultures are in need of frequent transfers to fresh agar media in order to keep them going and to prevent them from dying out.

Raciborski (1900) was the first to describe this fungus from Java. Butler and Kulkarni (1913) conducted a detailed study of the same fungus from India. Their isolates produced sporangia, chlamydospores and oospores. The cultures now received from New Delhi were of the non-oospore forming type (non-fertile) and produced only sporangia and chlamydospores in single strain culture. The behaviour of this isolate when

TABLE VI

Statement showing the development of oospores in paired culture

Paired with		Results	
<i>P. palmivora</i> (Tomato) plus strain	..	Oospores formed in limited numbers in 7 days	
do (Jack) do	..	do	6 do
do (Betelvine) do	..	Few oospores in 9 days	
do (Coconut) minus strain	..	Numerous oospores in 5 days	
do (Hevea) do	..	do	4 do
do (Breadfruit) do	..	do	3 do
do (Agave) do	..	do	4 do
do (Jatropha) do	..	do	4 do
do (Spondias) do	..	do	4 do
do (Areca-tyagli) do	..	do	9 do
<i>P. parasitica</i>	..	do	4 do
do var. <i>piperina</i>	..	do	6 do

grown in paired cultures with other isolates of *P. palmivora* and *P. parasitica* was studied and the results are shown above.

The behaviour of this isolate is akin to that of *P. parasitica* var. *piperina*. More oospores develop in combination with the minus strains of *P. palmivora* than with the plus strains. The studies of this fungus have shown that it closely resembles *P. parasitica* and *P. palmivora* in its morphological characters. The ease with which it pairs with these two species and forms oospores of the same type brings out the affinities between them more clearly.

The size of the sexual bodies observed by Butler and Kulkarni (1913) for *P. colocasia* agrees closely with those of oogonia and oospores formed in paired cultures. These are given below.

TABLE VII  
Statement showing the sizes of oogonia and oospores

Combination	Oogonia		Oospores	
	Mean $\mu$	Range $\mu$	Mean $\mu$	Range $\mu$
<i>P. colocasia</i> (Butler and Kulkarni)	29.5	24-31	23	20 -28
<i>P. colocasia</i> + <i>P. palmivora</i> (tomato)	26	24-28	21	18.5 -22
do + do (areca-tyagi)	31.5	25-41	24	18 -34
do + do (Spondias)	27.5	21-34	22	15.5-25
do + <i>P. parasitica</i>	28	24-31	22	18 -25
do + <i>P. parasitica</i> var. <i>piperina</i>	29.1	23-33	22.5	16 -25

The measurements are comparable to those recorded for sexual bodies formed in paired cultures of *P. palmivora*. In this isolate one can however detect an indication of the weakening of the homothallic tendency since it forms larger number of oospores with the minus strains of *P. palmivora* than with the plus strains. Probably this isolate will in course of time lose its capacity to combine with the plus strains and itself may behave as a plus strain. It may be interesting to note the behaviour of an isolate of *P. palmivora* from *Colocasia antiquorum* recorded by Thomas *et al.* (1947) which had the sporangial character usually attributed to *P. colocasia* but was non-oospore forming and behaved as a plus strain combining with the minus strains of *P. palmivora*. All these factors indicate the necessity for merging this species with *P. palmivora*. Leonian (1925) was the first to express a similar view.



6. *Phytophthora parasitica* Dast.

Mr. Dastur, the Head of the Division of Mycology, Indian Agricultural Research Institute, New Delhi, kindly sent us an authentic type culture of *P. parasitica* for our studies. This species was first described by Dastur (1913) from India having been isolated from blighted leaves of *Ricinus communis*. Since then it has been studied by numerous workers in different parts of the world on various hosts. The original isolate has been described as forming oospores in single strain cultures. But Tucker (1931) found that the "occurrence of oogonia and oospores in cultures is uncertain. They may appear only after several months or frequently not at all." The isolate obtained from New Delhi did not produce oospores at laboratory temperature, i.e., 24°–30° C. even after six months.

Paired cultures in combination with other isolates of *P. palmivora*, *P. colocasiae* and *P. parasitica* var. *piperina* were grown and the results are recorded below.

TABLE VIII

*Statement showing the development of oospores in paired cultures*

Paired with isolate			Results		
<i>P. palmivora</i>	Tomato plus strain	..	Oospores	numerous in	7 days
do	<i>Colocasia</i> do	..	do	formed in	8 do
do	Jack do	..	do	do	5 do
do	Betelvine do	..	do	do	10 do
do	Citrus 1 do	..	do	do	10 do
do	Arecas do	..	do	do	10 do
do	<i>Clerodendron</i> do (new)	..	do	numerous in	4 do
do	Coconut minus strain	..	do	formed in	10 do
do	Agave do (new)	..	do	numerous in	4 do
do	<i>Spondias</i> do (new)	..	do	do	4 do
do	<i>Hevea</i> do	..	do	do	4 do
do	Breadfruit do (new)	..	do	do	4 do
<i>P. colocasiae</i>		..	do	many	10 do
<i>P. parasitica</i> var. <i>piperina</i>		..	do	do	4 do

The isolate combines readily with heterothallic and homothallic isolates of *Phytophthora*. It forms oospores with fertile as well as non fertile homothallic strains. But when grown in single strain cultures oospores are not formed. Further, when this isolate is paired with an isolate of *P. palmivora* from *Spondias mangifera* which had become neutral no oospores developed even after a month. This demonstrates the fact that oospore formation in paired cultures is governed by the biological nature of the isolates that are brought together and the neutral strains do not pair at all.

The size of the sexual bodies produced by this isolate in paired culture falls within the range recorded for *P. palmivora* or *P. parasitica* as shown below.

TABLE IX  
Measurements of sexual bodies produced by *P. parasitica*  
with other isolates of *Phytophthora*

Particulars	Oogonia		Oospores	
	Mean $\mu$	Range $\mu$	Mean $\mu$	Range $\mu$
<i>P. parasitica</i> + <i>P. palmivora</i> (tomato)	26	21-31	20	15-22
do do (citrus 1)	27	24-34	21	15-28
do do ( <i>Jatropha</i> )	29	24-37	22	18-29
do do (Coconut)	28	25-32	22	21-25
do <i>P. colocasia</i>	28	24-31	22	18-20
do <i>P. parasitica</i> var. <i>piperina</i>	28	21-34	23	16-25

The range of measurements of the oogonia of *P. parasitica* on agar media is 15-35  $\mu$  and of oospores 13-26  $\mu$ . In some strains the oospores "which mostly fill the oogonial cavity range from 12  $\mu$  to over 35  $\mu$ " (Tucker, 1931). The sizes of oogonia and oospores formed in homothallic or paired cultures of *P. palmivora* range from 16 to 39  $\mu$  and 12 to 28  $\mu$  respectively.

The two allegedly different species have almost identical ranges of size of sexual bodies and the oospores formed in the paired cultures included in these studies also fall within this range.

### DISCUSSION

The results of the studies detailed above show the necessity for the revision of the current taxonomic classification of these isolates. The different isolates studied are at present grouped into *P. colocasia*, *P. palmivora*, *P. parasitica* and *P. parasitica* var. *piperina*. The criteria influencing the determination of species of *Phytophthora* include the nature of growth on culture media, temperature relations, size and shape of sporangia and chlamydospores, the readiness with which sexual bodies are formed, their size, the nature of the antheridia and pathogenicity of the isolates. We shall consider these factors with reference to the isolates under study and see how far they can be relied upon.

The nature of growth of different isolates on agar media exhibit certain differences. But these differences cannot be relied upon for specific differentiation. Some isolates of *P. parasitica* and *P. palmivora* exhibit almost

identical growths of aerial mycelium on oat agar and could not be distinguished from one another. Leonian (1925) has also found that his observations afforded no justification for the practice of making the production of aerial hyphae a distinctly specific character. Numerous intergradations in the amount of aerial hyphae may be produced by the strains of a single species. Hence this character cannot be relied upon. Tucker (1931) also found that the same single strain culture of *P. parasitica* may produce various types of growth on different media.

The same author has stated that the main difference between *P. parasitica* and *P. palmivora* is in their temperature relationship. The same differential behaviour is cited between *P. colocasiae* and *P. parasitica* also. He found that *P. parasitica* grew on cornmeal agar at 35° C. while *P. palmivora* and *P. colocasiae* did not. He further states that "the use of the ability to grow at certain temperatures as a criterion for the identification of species seems to be justified." Leonian (1934) in his studies on temperature relationships of many species of *Phytophthora* found that in both *P. parasitica* and *P. palmivora* some isolates grew at 35° C. while others did not. Mehrlich (1936) found that temperature relations for separating *P. parasitica* and *P. palmivora* do not hold good for isolates of *P. parasitica* from heart-rot of pine-apples. Six isolates of *P. parasitica* did not grow at 35° C. while four others did and all of them were morphologically alike.

In order to verify the behaviour of the local isolates one type culture of each of *P. colocasiae*, *P. palmivora* and *P. parasitica* was inoculated in triplicate on oat agar in petri-dishes and kept in an incubator at 35° C.  $\pm \frac{1}{2}$ ° C. When examined on the fifth day, all the three exhibited growth, the largest diameter being in *P. parasitica* and next in *P. palmivora* and still less in *P. colocasiae*. Though there were differences in the amount of growth all of them had grown. Thus the ability to grow at 35° C. is exhibited by all the three isolates. Hence this reaction cannot be utilised for taxonomic purposes. Further the isolates under study are all tropical organisms and 35° C. is a temperature to which they will be exposed during certain parts of the year and consequently they must have become acclimatised to such high temperatures. The culture of the local isolate from castor can be kept alive for a long period. But the type culture of *P. parasitica* from Delhi died out in a few months under local conditions. It is, however, shown that the differences observed by Tucker between these three species does not always hold good.

Sporangial characters have been overemphasized by a number of mycologists in differentiating species of *Phytophthora*. *P. colocasiae* is said to be

easily distinguished from other allied species by its elongated narrowly ovate sporangia which are shed with remnants of the pedicel attached to the base of the sporangium. When cultures of *P. colocasiae* are examined these features are not found to be constant. Broadly oval sporangia as in *P. parasitica* or *P. palmivora* are also formed. Further some isolates of *P. palmivora* develop elongated sporangia resembling those of *P. colocasiae* (Leonian, 1936; Thomas *et al.*, 1947). The size and shape of the sporangia in these three species are so variable and unstable that an undue emphasis should not be laid on these characters for specific differentiation. There are no constant differences between the sporangia of the three species.

The same may be said about the chlamydospores. These bodies are produced in varying numbers by the different isolates of these three species and no specific difference could be made out between them.

Considering the development of the oogonia and oospores it has been found that all the three species have been known to form these bodies in single strain cultures and no differences exist between them regarding the size attained by these bodies. The antheridium in all the three species is amphigynous. *P. colocasiae* and *P. parasitica* were distinguished from *P. palmivora* by the readiness with which oospores were formed in single strain cultures of the two former species. Some isolates of *P. meadii* which is now considered as *P. palmivora* formed oospores readily. The studies recorded above have further shown that depending on the isolates all the three species are capable of forming oospores in single strain cultures. It has also been found that in all the three species non-oospore forming cultures are available. The isolates are non-oospore forming either soon after fresh isolation or after some generations on agar media. Other workers have also found (Tucker, 1931) that the occurrence of oogonia and oospores in culture in *P. parasitica* is uncertain. "Seldom do they appear promptly and frequently not at all." Thus this character cannot be relied upon and no difference is seen between the three species in the size or shape of oogonia, oospores or antheridia, when these are developed.

Pathogenicity has been given an undue importance in delimiting species. Though this character may be of value in obligate parasites, when dealing with organisms, like species of *Phytophthora* this method of differentiation is almost of no value for distinguishing species. *P. colocasiae* was thought to be confined to *Colocasia antiquorum* and this phenomenon was made much of. But Thompson (1929) and Thet Su (1938) have found *P. colocasiae* on *Piper betle*. Thomas *et al.* (1947) have isolated *P. palmivora* from *Colocasia antiquorum*. A consideration of the host range of *P. palmivora* and

*P. parasitica* in nature reveals that both are formed on most of the recorded host plants. Below are given some of the recorded hosts of the three species.

TABLE X

Statement showing some of the common recorded hosts of *P. palmivora*, *P. colocasiae* and *P. parasitica*

Host	Country where recorded	Identification of pathogen	Parts affected	Isolated by
1. <i>Citrus</i> spp. ..	Bombay	<i>P. palmivora</i>	Foot rot and fruit rot	Uppal
	Madras	do	Fruit rot	Thomas <i>et al.</i>
	Philippines Porto Rico	<i>P. parasitica</i> do	do	Reinking Dreschter
2. <i>Cocos nucifera</i> ..	Madras	<i>P. palmivora</i>	Bud rot	Shaw and Sundaraman
	Philippines	<i>P. parasitica</i>	do	Reinking
	Porto Rico	<i>P. palmivora</i>	do	Tucker
3. <i>Colocasia antiquorum</i>	India	<i>P. colocasiae</i>	Leaf spot and Blight	Butler and Kulkarni
	Madras	<i>P. palmivora</i>	do	Thomas <i>et al.</i>
	Java	<i>P. colocasiae</i>	do	Raciborski
4. <i>Gossypium barbadense</i>	Porto Rico	<i>P. parasitica</i>	Boll rot	Tacker
	St. Vincent	<i>P. palmivora</i>	do	Ashby
	Montserrat	do	do	Wakefield
5. <i>Lycopersicon esculentum</i>	New York	<i>P. parasitica</i>	Fruit rot	Redlick
	U.S.A.	do	do	Lavelle
	Madras	<i>P. palmivora</i>	do	Ramakrishna and Sowmini
6. <i>Piper betle</i> ..	Central Provinces (India)	<i>P. parasitica</i> var. <i>piperina</i>	Wilt	Dastur
	Bengal	<i>P. palmivora</i>	do	McRae
	Madras	do	do	Thomas <i>et al.</i>
	Malaya	<i>P. colocasiae</i>	do	Thompson
	Burma	do	do	Thet Su
7. <i>Solanum melongena</i>	Philippines	<i>P. palmivora</i>	Fruit rot do	Reinking
	do	<i>P. parasitica</i>		Ocfemia
	do	do		Reinking
8. <i>Theobroma cacao</i>	Java	<i>P. palmivora</i>	Pod rot	Ashby
	Ceylon	do	do	Gadd
	Surinam	<i>P. parasitica</i>	do	Stahel

The above list includes instances where the pathogen isolated from the same host affected by similar diseases has been differently named. The frequency with which *P. palmivora* and *P. parasitica* have been recorded on the same host plants denotes the identical pathogenic qualities of those two. It is surmised that the relative identification of the pathogen might have been influenced by the development of sexual bodies in cultures or their

absence. *P. colocasiae* is however restricted in its parasitism. For a long time it was known only on *Colocasia antiquorum* but in Malaya Thompson (1929) and in Burma Thet Su (1930) have recorded it on *Piper betle* also. Thomas *et al.* (1947) have isolated *P. palmivora* from leaves of *C. antiquorum*. Restricted parasitism of certain strains of the same species is well known in fungi. It is also known that the pathogenic ability of facultative suprophytes can be changed by slow 'education' of the particular strains. Therefore the exclusive use of pathogenicity to distinguish species among facultative saprophytes is not a reliable guide.

The close relationship between these species is further illustrated by the ease with which one is mistaken for the other. Thompson (1929) concluded from his studies of a number of isolates of *Phytophthora* that *P. parasitica* may develop homothallic and heterothallic strains and the latter can be regarded as being atypical members of *P. palmivora*. The tobacco fungus was originally described by Breda de Haan in 1896 as *P. nicotianæ*. Tisdale (1922) observed the close similarity between this and *P. parasitica* which was confirmed by Leonian (1925). Lester Smith (1927), however, observed that the size of the sporangium in *P. parasitica* agreed with that of *P. nicotianæ* but considered the latter to be a strain or form of *P. palmivora* from its behaviour in paired cultures with isolates of that species. Ashby (1928) included *P. nicotianæ* in *P. parasitica*. Tucker (1931) wanted to give a distinguishing name to the organism responsible for black shank of tobacco and named it *P. parasitica* var. *nicotianæ*. Thomas *et al.* (1947) found that the black shank organism could be classified as *P. palmivora* on account of its behaviour in paired cultures and the absence of any distinguishing characters to consider it as a separate species or variety. The frequent changes in the nomenclature of this fungus shows how the two species are very much alike and are liable to be interchanged according to the isolate under study.

The readiness with which the isolates at present classified as *P. colocasiae*, *P. palmivora* and *P. parasitica* combine in paired cultures and form oospores is a further proof of their close specific affinity. Lenonian (1931) followed the behaviour of 85 cultures of *Phytophthora* belonging to his 'Oomivora group'. The formation of oospores in paired cultures by 48 of these is considered by him and rightly too as due to heterothallism and not hybridization between species. If we are to consider the pairing between *P. parasitica* and *P. palmivora* as one of hybridization as believed to be by Narasimhan (1930) there must be some features in the resulting oospores which distinguish them from those formed when two heterothallic isolates of *P. palmivora* are paired. Narasimhan took this view owing to the size

of the oospores formed when *P. parasitica* was paired with *P. palmivora* which he thought was intermediate between those of the oospores of the two species. But it is now known that the size of oospores obtained by Narasimhan in his paired cultures is even obtained in single strain cultures of *P. palmivora* or of *P. parasitica*. Therefore his reasoning that hybridization has taken place in his cultures is not tenable. The formation of oospores in paired cultures of the isolates included in these studies has to be considered as union between heterothallic forms of the same species and not as instances of hybridization between different species.

It may be mentioned here that 'plus' and 'minus' strains of *P. palmivora* were grown with *P. cambivora* and *P. cactorum*. Oospores did not develop in the cultures with the former species. In the cultures with the latter species oospores were formed but they were of the '*Cactorum*' species with paragynous antheridia in the majority of sexual bodies.

The results of these investigations point to the desirability of amalgamating *P. parasitica*, *P. palmivora*, *P. colocasiae* and *P. parasitica* var. *piperina* into one species as there are no valid, reliable or constant differences between them to keep them separate. Leonian (1925) has already suggested the combination of all these under the binomial *P. omnivora*. This was objected to by Ashby (1928) who pointed out that the sporangia of *P. colocasiae* are distinguishable from those of *P. parasitica* and the ready development of oospores separates it from *P. palmivora*. Later studies by various authors have shown that both these characters are not constant and are exhibited by the other species also. Another objection raised by Ashby (1928) is, in regard to the use of the binomial *P. omnivora*. The specific name '*omnivora*' was coined by De Bary without regard to the prior name of '*cactorum*' used to denote the same species (Fitzpatrick, 1930). Therefore the name *P. omnivora* becomes a synonym of *P. cactorum* and cannot be revived to include the three species as intended by Leonian. Tucker (1931) has considered *P. omnivora* as a synonym of *P. cactorum*. In a later publication Leonian (1934) has suggested the merging of *P. parasitica* and *P. palmivora* under the latter species keeping *P. colocasiae* as a separate species. Our studies support his original view that all the three species should be merged into one. The obvious question which arises is, which is the name to be retained for the species in which three old species are merged. The correct procedure according to the rules of nomenclature is to adopt the oldest name, i.e., *P. colocasiae*. Dr. Bisby who was consulted about the specific name to be adopted held the view that if we were satisfied about the necessity for merging all the three species into one, the specific name '*colocasiae*' ought to be retained as it is the oldest and sufficiently well known. Accepting

Dr. Bisby's view it is proposed to combine *P. colocasiae*, *P. palmivora* and *P. parasitica* into one species *P. colocasiae*.

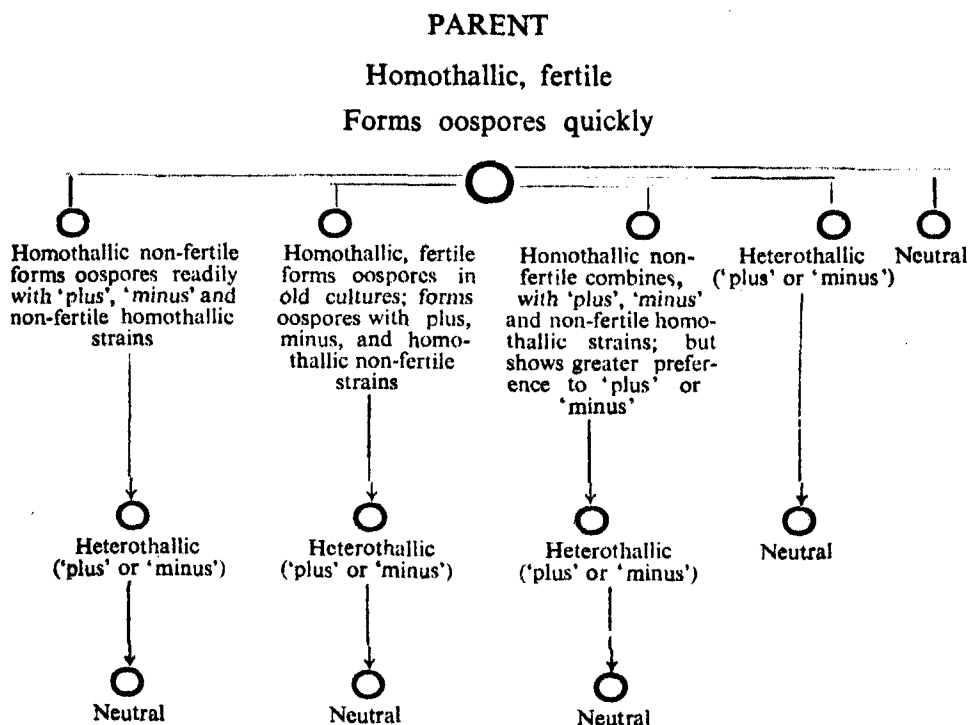
The behaviour of the isolates of this emended species in single strain and paired cultures is variable. Some isolates are fertile and produce oogonia and oospores in single strain cultures quickly. Other isolates form these bodies very late. Both these may be described as homothallic and fertile. Still other isolates do not form oospores in single strains but combine readily with the plus and minus strains. These may be considered as homothallic but non-fertile and require the sexual stimulation of another biologically active isolate to form oospores. Two homothallic but non-fertile forms also are capable of combination as the isolates of *P. colocasiae* and *P. parasitica* under study. Variations from this reaction are exhibited by some isolates which produce more oospores with the 'plus' strain than with the 'minus' strain or *vice versa*. Since neutral isolates do not form oospores in combination with homothallic non-fertile isolates the development of oospores must be considered to be governed by biological (sexual) nature of the isolate and not by the biochemical stimulation by the presence of any other isolate. Such homothallic non-fertile strains which exhibit a preference for the 'plus' or 'minus' strain might gradually give rise to the heterothallic strains of the 'minus' or 'plus' types themselves. With continued growth on agar media some of these become neutral, and do not form oospores with any combination of isolates. Thus from an original fertile homothallic isolate, non-fertile homothallic or heterothallic or neutral strains may be developed in course of time. This might represent the course of development of the sexual behaviour of this species in nature also with the result that different isolates behave in different ways according to their genetic make up. These changes can be attributed to new combinations, and segregations that occur during and after the sexual reproduction in *Phytophthora*. The germination of the oospore is usually accompanied by the meiotic division of the fertilised nucleus and this feature must account for the segregation of factors and formation of new races and strains. Mutations occurring during the life of the isolate in pure cultures lead to further variations.

Edgerton *et al.* (1944) have noticed during their studies on the genetics of *Glomerella* that perithecia-forming strains, 'plus' strains and 'minus' strains exist in the isolates of the fungus. Perithecia-forming strains may combine with 'plus' or 'minus' strains leading to the formation of larger number of perithecia. They have also seen that a 'plus' strain may give rise to a 'minus' strain or perithecia producing strain. Chilton and Wheeler (1947) are of opinion that new strains of *Glomerella* arise by mutation.



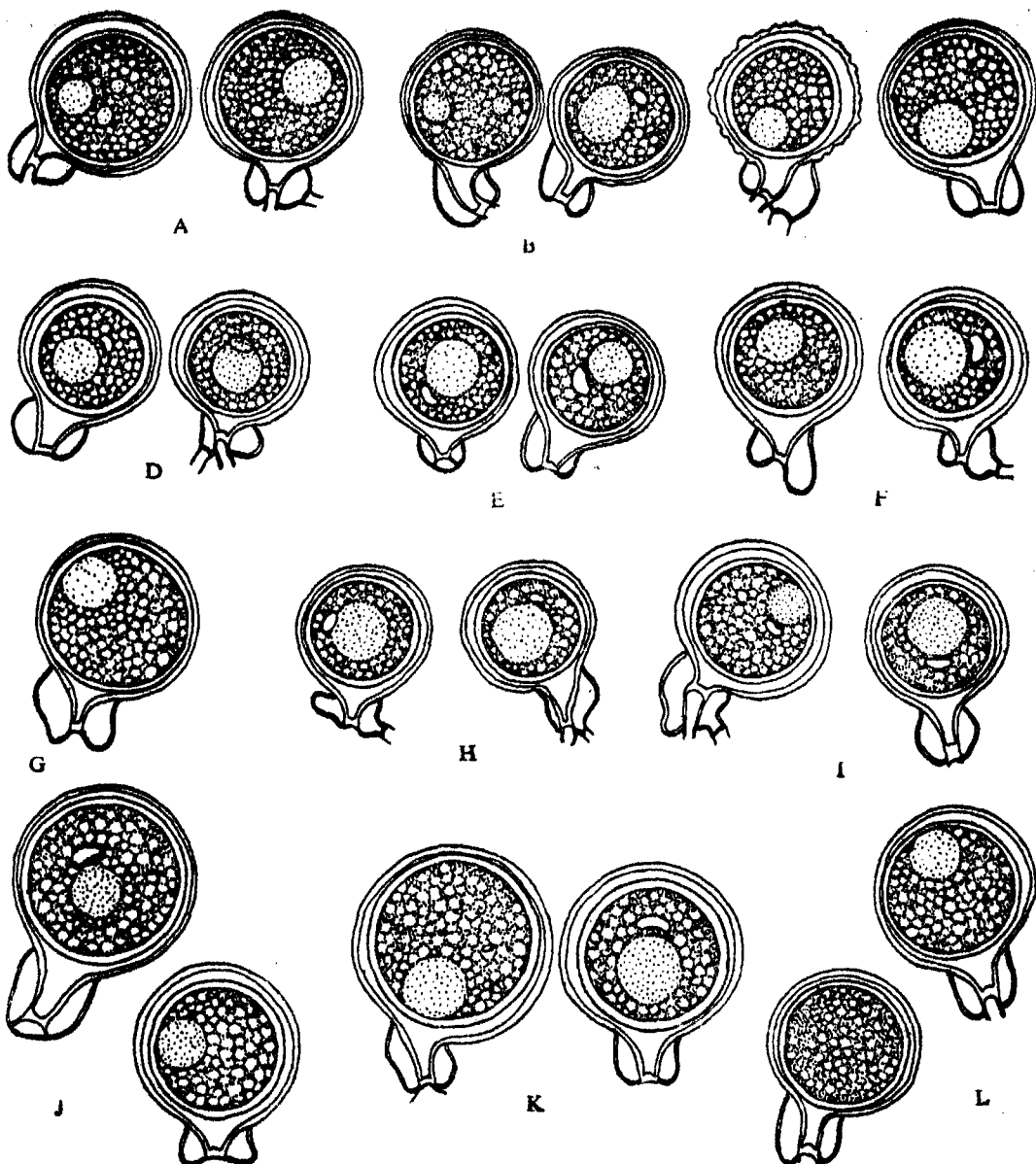
from the 'plus' strain. The above is comparable to what takes place in the cultures of *P. colocasiae*.

Speculation on the origin of the different isolates studied is not attempted but a suggestion is put forward indicating the possible course of development of the different strains as shown below:



Leonian (1934) has emended the description of *P. palmivora* (including *P. parasitica* and *P. palmivora*). With slight alternations the description for the emended species of *P. colocasiae* (Racib.) Thom. and Ram. now proposed will read as follows:—

The emended species of *Phytophthora colocasiae* (Racib.) Thom. and Ram. (merging *P. colocasiae*, *P. palmivora*, *P. parasitica* and its varieties) enjoys a world-wide distribution and has been recorded on numerous host plants thus exhibiting an omnivorous habit. The growths of this fungus on agar media exhibit wide variation. Aerial hyphae may be luxuriant, scanty or sometimes absent; sub-merged hyphae, smooth or gnarled, even or uneven; the temperature tolerance varies according to the country of origin of the isolate, from 30° C. to 37·5° C. Sporangia distinctly papillate, greatly variable in size and shape; chlamydospores spherical



The drawings of oospores have been made with the aid of an Abbe camera lucida at uniform magnification of  $\times 680$ .

Oospores formed in paired cultures of different combinations :—

A.	<i>P. palmivora</i>	(plus, Tomato)	$\times$	<i>P. parasitica</i> var. <i>piperina</i>
B.	do	(minus, Areca)	$\times$	<i>P. parasitica</i>
C.	do	(minus, Coconut)	$\times$	do var. <i>piperina</i>
D.	do	(plus, Tomato)	$\times$	<i>P. parasitica</i>
E.	<i>P. colocasia</i>		$\times$	do
F.	<i>P. palmivora</i>	(minus, <i>Jatropha</i> )	$\times$	do
G.	do	(minus, Coconut)	$\times$	do
H.	do	(plus, Citrus)	$\times$	do
I.	do	(plus, Betelvine)	$\times$	do
J.	do	(minus, Coconut)	$\times$	<i>P. colocasia</i>
K.	do	(minus, Areca)	$\times$	do
L.	do	(plus, Tomato)	$\times$	do

or subspherical, light or deep coloured, terminal or intercalary; some isolates may produce more of these bodies than others. Sexual bodies present or absent: heterothallic, homothallic and neutral strains present; antheridia typically amphigynous; size of oogonia and oospores greatly variable, oogonia 13–41  $\mu$ , oospores 12–35  $\mu$  in diameter.

We wish to express our indebtedness to Dr. Asthana of Nagpur and Mr. J. F. Dastur, Head of the Division of Mycology, New Delhi, for kindly supplying type cultures of certain isolates. To Dr. Bisby of the Imperial Mycological Institute, Kew, we are thankful for the advice on the choice of specific name. Mr. M. S. Balakrishnan helped us in isolating some of the strains and in making the diagrams. Miss C. K. Soumini made the measurements of oogonia and oospores in some of the paired cultures. We offer our thanks to them for the help rendered.

#### SUMMARY

Isolates of *Phytophthora* from castor, *Agave*, and breadfruit and authentic cultures of *P. parasitica*, *P. parasitica* var. *piperina* and *P. colocasiae* were studied in detail in single strain cultures and in paired cultures in different combinations.

It was found that the isolates from the different hosts under study easily combined with *P. parasitica*, *P. parasitica* var. *piperina* and *P. colocasiae*, forming oospores. These oospores are of the same type and fall within the range of size recorded for *P. palmivora* and *P. parasitica*. The criteria on which the species under investigation are classified are critically examined and it is found that no significant differences exist between them. The three species are able to grow at 35° C.

The readiness with which these combine to form oospores shows their specific affinity. It is argued that all the three species should be combined into one and the name *P. colocasiae* is adopted for the emended species as it is the oldest.

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# CYTOLOGY OF *COCCINIA INDICA* W. & A. WITH REFERENCE TO THE BEHAVIOUR OF ITS SEX-CHROMOSOMES

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## I. INTRODUCTION

THE family Cucurbitaceæ includes quite a large number of diœcious species. Perhaps on this account it received the attention of the cytologists as early as 1907 when Kirkwood (1907) studied the microsporogenesis of *Micrampelis alba*. Strasburger (1910) first worked on *Bryonia alba* with a view to investigating the mechanism of the diœcious nature of the plant. Sinoto (1928) had observed the presence of a pair of heterochromosomes in *Trichosanthes japonica*; but Bönicke (1911), Lundergardh (1914), Meurman (1925) and Lindsay (1930), working independently with *Bryonia dioica*, failed to find the presence of any heteromorphic pair of chromosomes in this species. Banerji and Das (1937), while studying the meiosis of *Trichosanthes dioica*, did not observe any sex-chromosome pair nor did they obtain any evidence in that direction from a study of somatic mitosis. Nakajima (1937) reinvestigated the meiotic process of *Trichosanthes japonica* and found the presence of a pair of sex-chromosomes as was previously observed by Sinoto (1928). He also recorded the occurrence of a pair of heteromorphic chromosomes in *T. cucumeroides*. Kumar and Deodikar (1940) recorded their observations on the cytology of *Coccinia indica*. They found 26 chromosomes in the somatic nuclei of both male and female plants. Of these, one pair was found to be homomorphic (XX) in the male and heteromorphic in the female (XY). During the meiotic division of the PMCs also, they could observe the presence of a homomorphic pair of chromosomes and noted its behaviour during meiosis. The chromosome number of *C. indica* was previously determined to be  $n = 12$  by Sutaria (1936). He, however, had not stated anything about the presence of a pair of sex-chromosomes in this species. It may be pointed out that the chromosome number of the closely allied species *C. hirtella* is also  $2n = 24$  (cf. McKay, 1930).

A preliminary examination of the somatic nuclei of both the sexes of *C. indica* revealed certain interesting features which did not agree at all with those previously recorded by Kumar and Deodikar (1940). It was found

that the somatic chromosome number of this species as determined by Sutaria (1936) is  $2n = 24$  instead of  $2n = 26$  as claimed by Kumar and Deodikar. Further, the nature and combinations of the sex-chromosome pair in which they occur in the two sexes were found to be just the reverse of what had been observed by Kumar and Deodikar, i.e., it was found to occur in a heteromorphic (XY) combination in the male and homomorphic (YY) in the female (cf. also Bhaduri and Bose, 1947).

From the facts stated above it becomes apparent that a thorough study of the different stages of mitosis and meiosis in both the sexes of *C. indica* is more than necessary in order to arrive at a definite conclusion with regard to the mechanism of sex differentiation in this species. The present paper therefore deals chiefly with the behaviour of the pair of sex-chromosomes at different stages of mitosis in the root-tip cells of both sexes and at the stages of meiosis in the male sex alone of *C. indica*.

## II. MATERIAL AND METHODS

The material used in this investigation was collected from plants of *C. indica* growing wild in the Calcutta University College Garden. For the study of somatic mitosis, adventitious roots of both male and female plants, which were obtained from the nodes of scrambling stems, were fixed in La Cour's 2BE fluid which proved to be suitable for the purpose.

For the study of meiosis, flower buds of the male plants were fixed in Nawashin's fluid and Flemming's fluid both of which yielded good results.

After fixation the materials were washed, dehydrated and cleared in the customary way. Sections were cut 8 to  $12\mu$  thick, depending on the stage required for study. Heidenhain's Iron Alum Haematoxylin and Newton's Iodine Gentian-Violet stains were chiefly used.

Observations were made with the help of a compensating eyepiece ( $\times 10$ ) and an apochromatic objective (1.2 mm.). Figs. 11 to 14 were drawn at a table magnification of  $\times 1,600$ . All other drawings were made with a  $\times 20$  compensating eyepiece and an 1.2 mm. apochromatic objective, giving a magnification of  $\times 3,700$  at the level of the table.

## III. OBSERVATIONS

### 1. Somatic mitosis

A study of the somatic chromosomes of both the sexes of *C. indica* revealed that the nuclei of the cells composing the tissue of the male plant are comparatively smaller in size and possess a heteromorphic pair of chromosomes which is absent in the female. In other respects, however, the nuclear

behaviour in somatic mitosis appears to be similar in both the sexes. Hence a common account is presented below, the points of difference being mentioned at the appropriate places.

*Resting nucleus*—The resting nucleus is comparatively large. Nothing chromatic in nature is visible within the nucleus by the ordinary technique applied except a large nucleolus. The karyolymph however, is not homogeneous. It forms two distinct concentric zones. The zone around the nucleolus is transparent and colourless. The second zone, just outside the first and reaching upto the nuclear membrane, is slightly darker. The nucleolus shows a distinct bud-like process.

*Prophase*—With the commencement of prophase, small and irregular chromatic bodies are evident in the outer zone of the nucleus (Fig. 1). The size and chromaticity of these bodies gradually increase and they finally assume the structure of typical chromosomes each of which is composed of two intertwining threads. An interesting feature observed at this stage is the presence of a large chromosome in the nuclei of the male plant (Fig. 2, chromosome X). This is absent in the nuclei of the female plant (Fig. 3).

When the nucleus and the chromosomes reach their maximum size, the former gradually shrinks inwards from the two poles, leaving behind two 'polar caps' of spindle substance (Fig. 4). As the nuclear membrane shrinks more and more around the crowded chromosomes, the 'caps' become larger and larger and finally assume a definite spindle-shaped figure in which the spindle fibres originate. The double nature of the chromosomes is less evident at this stage due to the increased chromaticity of the chromosomes. Eventually, the nuclear membrane disappears and most of the chromosomes aggregate around the irregular nucleolus which, by this time, has become much reduced in size.

*Prometaphase*—Prometaphase begins with the disappearance of the nuclear membrane and the organization of the spindle from the 'polar caps'. At this stage the chromosomes condense further and their chromaticity also increases with the result that the double nature of the chromosomes does not become apparent. Soon the nucleolus disappears and the chromosomes lie irregularly scattered over the spindle. At this stage the chromosomes are found to have undergone maximum condensation. Their intertwining nature no longer persists and the two chromatids of each chromosome lie parallel with one another and are seen to be smoother in contour.

*Metaphase*—The metaphase begins with the orientation of the chromosomes in the equatorial plane of the spindle. Associated with this orienta-

tion the individuality of the two chromatids of a chromosome is no longer evident. A polar view of the metaphase plate shows 24 chromosomes in the preparations of root tips of both male and female plants. But as has been stated before, in the male plant there is a very large chromosome, compared to others all of which are more or less of the same size (Figs. 5 and 6). In the female plant, on the contrary, all the 24 chromosomes are nearly of the same size (Fig. 7).

*Anaphase*—The movement of the chromosomes is regular and simultaneous (Fig. 8). The two chromatids of each chromosome move to the opposite poles of the spindle where they form the polar clumps (Fig. 9). Due to the compactness of the polar clumps the individuality of the chromosomes at this stage is lost altogether.

*Telophase*—Telophase begins with the relaxation of the polar clumps from which the chromosomes emerge out. The chromosomes then appear in the form of slender threads which are thick at certain regions (Fig. 10). They are very soon enclosed by a nuclear membrane. The chromosomal threads gradually become thinner and their chromaticity decreases. Soon the nucleoli make their appearance in each daughter nucleus. Gradually the chromosomes lose their chromaticity and the nucleus appears to be homogeneous except for the large nucleolus. The nucleus at the same time grows bigger in size and assumes the structure described under the resting nucleus.

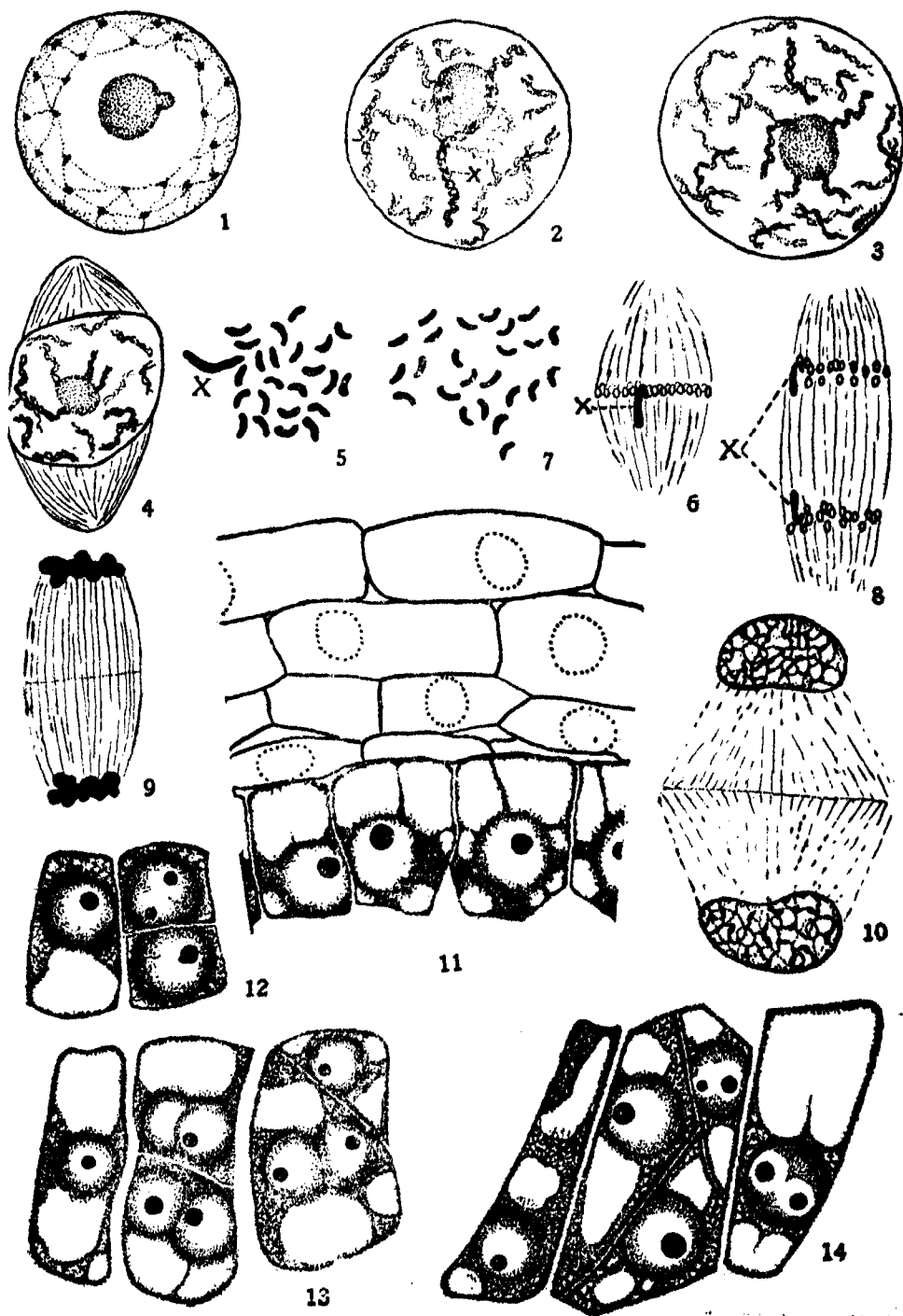
## 2. *Tapetum*

The archesporial cells which are hypodermal in origin cut off periclinally a primary parietal layer just below the epidermis and the primary sporogenous cells on the inner side. The primary parietal layer then divides by periclinal walls and forms four layers of cells. Of these, the outermost layer which lies just below the epidermis develops into the endothecium and the innermost one differentiates as the tapetal layer (Fig. 11).

The tapetal cells, to begin with, possess a comparatively conspicuous and large nucleus and deeply staining cytoplasm with large vacuoles (Fig. 11).

During the synizesis of the pollen-mother cells, the nucleus of the tapetal cell divides once only, but this division, as a rule, is not followed by wall formation. Consequently, the two nuclei remain free in the cell. This binucleate condition of the tapetal cells is maintained throughout the development of the pollen grains. In no case has further division or fusion of these nuclei been observed.





FIGS. 1-14

FIGS. 1-14.—Figs. 1-10. Stages in somatic mitosis in the root-tip cells of *C. indica*. Fig. 1. Early prophase. Figs. 2 and 3. Late prophase nuclei of male and female plants. Note the chromosome X in Fig. 2. Fig. 4. Polar caps. Fig. 5. Metaphase plate of male plant. Note the chromosome X. Fig. 6. Metaphase of the same in side view. Fig. 7. Metaphase plate of female plant. Fig. 8. Anaphase of male plant showing the chromosome X. Fig. 9. Polar clump. Fig. 10. Telophase. Fig. 11. Uninucleate tapetal cells. Fig. 12. Two tapetal cells in one of which nuclear division has been followed by cytokinesis. Figs. 13 and 14. Different portions of tapetum showing different characters of individual tapetal cells.

It is interesting to note that in a few tapetal cells nuclear division is followed by cytokinesis leading to the formation of two uninucleate cells (Fig. 12). Further divisions of these cells sometimes take place giving rise to binucleate daughter cells or uninucleate grand-daughter cells as will be seen from Figs. 13 and 14.

Cooper (1933) after a thorough study of the nuclear behaviour in tapetal cells of 7 monocotyledonous and 36 dicotyledonous species has formulated three main groups based on the nuclear phenomenon of tapetal cells. These are as follows:—

*Group I*—in which the mature tapetal cell is uninucleate,

*Group II*—in which the nucleus divides once only mitotically and the binucleate condition is maintained throughout the development of the pollen grains,

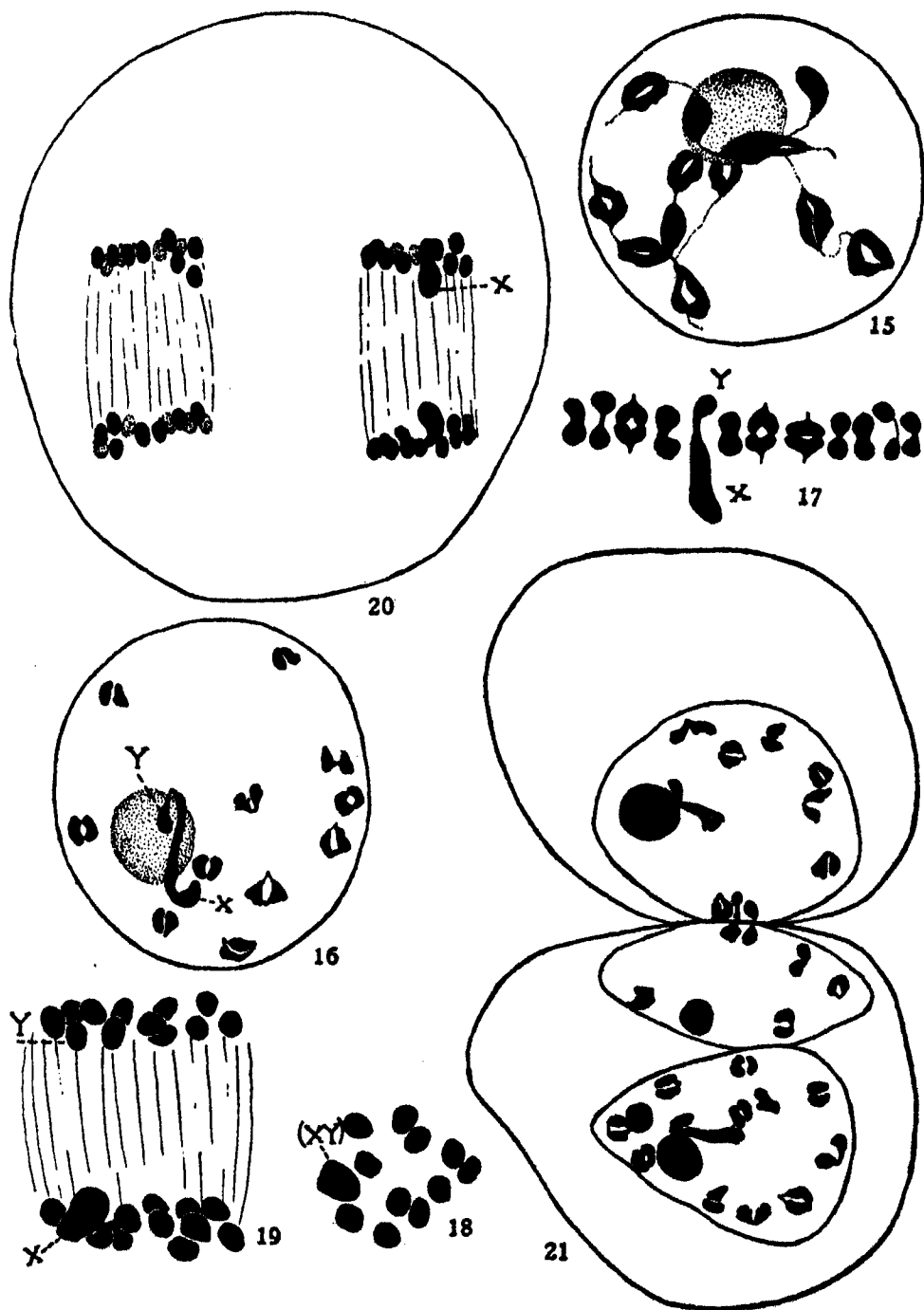
*Group III*—in which the cells are multinucleate as a result of more than one mitosis. In this group, those plants in which the cells remain uninucleate or binucleate through abnormality in divisions, are also included.

Referring to the above classification it appears that *Coccinia indica*, strictly speaking, does not belong to any of these three groups because the divisions of nuclei of some of its tapetal cells are followed by cytokinesis (Figs. 12 to 14), a condition which has not been recorded by Cooper (1933) and is not found in any of the three groups formulated by him. Nevertheless it shows resemblance to Group II more closely than to the other two, as the tapetal cells or their derivatives attain the binucleate condition by a single mitosis and which condition is retained thereafter.

The two nuclei in the tapetal cell are at first somewhat apart from each other, but soon they come quite close and lie either side by side or one above the other. This approximation of the two nuclei is sometimes so close that they may lie in actual contact with one another (Fig. 13).

### 3. *The behaviour of chromosomes during meiosis*

The earlier stages of meiosis pass through quite regularly. The presence of the sex-chromosome pair could not be observed until the nuclei



FIGS. 15-21

FIGS. 15-21. Stages of meiosis in PMCs of *C. indica*. Fig. 15. Early diakinesis. Fig. 16. Typical diakinesis showing the heteromorphic pair (XY) attached to the nucleolus. Fig. 17. Side view of metaphase I showing XY pair. Fig. 18. Polar view of metaphase I. Fig. 19. Anaphase I showing disjunction of XY. Fig. 20. Anaphase II. Fig. 21. Binucleate PMC and cytomixis.

reached the diakinetik stage. As such the behaviour of the chromosomes have been described from diakinesis onwards.

*Diakinesis*—The diplonema threads open out a little and shortly afterwards the bivalents become organised. As the chromosomes shorten and thicken the chromatic elements of the diplonema threads appear to retract from the two ends and condense at the centre leaving behind achromatic threads which later disappear. Fig. 15 shows twelve such condensed masses of chromatic matter which represent the haploid number of chromosomes of the plant. Gradually the chromosomes condense still further and the linin connections between them become obscured. At this stage the twelve bivalents are visible in the nuclear cavity; of these one pair is distinctly heteromorphic a member of which is much larger than any of the members of the homomorphic pairs. The heteromorphic pair in most of the nuclei has been found to be associated with the nucleolus (Fig. 16).

*Metaphase I*—The bivalents take their position at the equatorial plane of the spindle (Fig. 17). A polar view just before anaphasic separation does no longer exhibit the double nature of the bivalents which assume a more or less rounded form. Among the twelve bivalents, one is distinctly larger than the rest (Fig. 18). The largest one evidently represents the heteromorphic pair observed in diakinesis.

*Anaphase I*—Anaphasic separation of the univalents begins soon. Some of them assume V-shaped forms, thereby suggesting that these have a median spindle attachment region. The heteromorphic pair becomes clearly marked out on account of the unequal size of its members, as will be seen from Figs. 17 and 19. The movement of the chromosomes is more or less regular and simultaneous. Often one or more univalents are seen to be cast off from the spindle. The univalents on reaching the two opposite poles form the polar clumps in which the identity of the chromosomes is lost. Later the chromosomes emerge out from the polar clumps as long threads and form two interkinetic nuclei.

*Division II*—The two interkinetic nuclei lose their membranes and their nucleoli disappear. Two bipolar spindles are organised and the two haploid groups of chromosomes which showed evidence of longitudinal splitting in the interkinetic nuclei arrange themselves at the equatorial region of the spindles. At this stage their longitudinal split becomes obscured.

Anaphasic movement soon commences and this is simultaneous in both the spindles. On reaching the poles the chromosomes lose their identity. Very soon a nuclear membrane is organised and the nucleoli make their appearance.

In one of the spindles of the second division the presence of the largest chromosome could easily be made out, but the presence of its homologue in the other could not be determined due to the almost equal size of the chromosomes of the spindle in question. Fig. 20 shows the two spindles during anaphase II. In one of these the presence of the largest chromosome may be noted.

The four nuclei formed as a result of the second division thus differ both qualitatively and quantitatively. Two of the nuclei which receive one longitudinal half of the largest chromosome are alike, so far as this chromosome is concerned, but differ from the other two which are again alike in possessing one longitudinal half of the homologue of the largest chromosome.

*Cytokinesis*—Cytokinesis takes place by furrowing. The arrangement of the pollen quartets is generally tetrahedral but isobilateral tetrads have also been noted. Quadripartition of the microspores by furrowing appears to be the rule in Cucurbitaceæ (cf. Asana and Sutaria, 1932; Castetter, 1926; Heimlich, 1927; Banerji and Das, 1937; Passmore, 1930).

#### 4. Irregularities during meiosis

The following abnormalities have been observed during the meiotic division of the pollen mother cells in *Coccinia indica*.

(i) *Cytomixis*—The phenomenon of cytomixis was first described by Gates (1911) while studying the microsporogenesis of *Oenothera gigas*. Katterman (1933) recorded the various stages of cytomixis in PMCs of *Triticum* × *Secale* hybrids. The same phenomenon was observed by Nandi (1937) in a variety of rice during the stages of meiosis and also by Banerji and Das (1937) in *Trichosanthes dioica*. Binucleate pollen mother cells have been noted by Gates and Rees (1921) in *Lactuca*, Karpechenko (1927) in the hybrids of *Raphanus* × *Brassica* and Kihara and Lilienfeld (1934) in certain hybrids between *Triticum* × *Aegilops*. Banerji (1940) working on *Tridax procumbens* observed multinucleate pollen mother cells.

In *Coccinia indica* binucleate pollen mother cells have been observed at almost every stage of development. The origin of these cells might be due to the union of cytoplasm of two pollen-mother cells or to a process of cytomixis or by the absence of wall formation during premeiotic division. In

all probability the last explanation seems to be more plausible but the occurrence of cytomixis is also not uncommon in this species. Fig. 21 illustrates a condition where one of the pollen mother cells contains two nuclei in diakinetik stage and the other only one such nucleus. It will be evident from this figure that an actual transference of chromatin is taking place between them. A similar condition has been figured by Banerji and Das (1937) in *Trichosanthes dioica*.

(ii) *Polyspory*—It is a common feature of *Coccinia indica* that, during the first meiotic division, one or more univalents are cast off from the spindle during anaphase. The cast-off chromosomes then organize a separate small nucleus in the cytoplasm of the pollen-mother cell. Hence in such pollen-mother cells three interkinetic nuclei are formed after the first division. Each of these nuclei divides again. The six resulting nuclei in the pollen-mother cell after cytokinesis give rise to six pollen grains, but the two developed from the cast-off chromosomes are much smaller in size. Such abnormality in pollen grain development has been recorded by a number of workers and Stebbins (1932) is of opinion that the irregularities in meiosis which lead to polyspory are due to heterozygosity.

#### IV. DISCUSSION

Among those who believed that sex determination is influenced by some internal conditions are Cuénot (1899) for animals and Strasburger (1900) for plants. The theory was subsequently strengthened by the discovery that in many animal and plant species the two sexes differ in their chromosome complements.

Since 1923, several species of diœcious angiosperms have been shown to be characterised by the possession of a heteromorphic sex-chromosome pair. Sinoto (1928) observed the behaviour of the sex-chromosomes in a number of diœcious monocotyledonous and dicotyledonous plants. Kihara (1929), Sinoto (1929) and Lindsay (1930) reviewed the subject of sex-chromosomes in higher plants and gave lists of diœcious plants whose sex-chromosomes had been studied. Nakajima (1937) observed the behaviour of the sex-chromosomes in 16 species of angiosperms. Besides these, a number of diœcious angiosperms have been studied with a view to determine the sexual mechanism involved.

It is well known that sex-chromosomes occur in male and female organisms in different combinations. In plants, these are mostly of the XY type as have been previously observed by Allen (1917, 1919) in *Sphærocarpus*, Heitz (1928 *a* and *b*) in *Pellia Neesiana* and by Santos (1923, 1924) in *Elodea canadensis*. Recently, a similar type of sex-chromosome pair

has been recorded in a number of angiosperms. Ono (1930) has observed the peculiar form of sex-chromosomes Y X Y or XYn type in *Rumex acetosa*.

*Coccinia indica* also shows XY type of sex-chromosomes. In connection with somatic mitosis it has been stated that the somatic chromosome number of both male and female plants is 24, of which one pair is heteromorphic in the male and obviously homomorphic in the female, although the homomorphic pair of the female could not be differentiated from the other chromosomes of the complement. But by a study of the behaviour of the unequal pair of chromosomes in the male plant during microsporogenesis and by a comparison of the size of the largest chromosome of the male plant with any one of the chromosomes of the female it can be definitely stated that the male contains 22 autosomes + XY and the female, 22 autosomes + YY.

It has been stated in connection with meiosis that two of the pollen grains of a quartet differ qualitatively from the other two, because two of them contain the X chromosome + 11 autosomes and the other two contain the Y chromosome + 11 autosomes. All the eggs produced by the female plant are qualitatively alike so far as the sex-chromosome is concerned, because each of them contains a Y chromosome + 11 autosomes. Hence the union between a male gamete developed from a pollen grain containing the X chromosome and an egg will produce a staminate plant and union of an egg with a male gamete developed from a pollen grain containing the Y chromosome will produce a pistillate plant.

Recently Kumar and Deodikar (1940) have made some observations on the somatic chromosome complement of both male and female plants and the behaviour of the sex-chromosomes during microsporogenesis of *Coccinia indica*. Their observations however, appear to be altogether different from those made during the course of the present investigation. According to them there are 26 chromosomes in the somatic nuclei of both male and female plants. Of these, one pair is homomorphic (XX) in the male and heteromorphic (XY) in the female. Hence according to them all the pollen grains or the male gametes are qualitatively alike and there are produced two kinds of eggs.

As regards the chromosome number of *Coccinia indica*, the author's observation agrees with that made by Sutaria (1936) and Bhaduri and Bose, (1947) who independently determined it to be  $n = 12$ . In this connection it may be mentioned that the somatic chromosome number of the closely related species, *C. hirtella*, is also  $2n = 24$  (cf., McKay, 1930).

As regards the origin of the sex-chromosomes of *Coccinia indica* during microsporogenesis, Kumar and Deodikar observed an interesting feature.

According to them the sex-chromosomes do not appear in the nuclear cavity until the autosomes undergo considerable longitudinal contraction and reach the late diplotene stage. They state, "From the persistent nucleolus an outgrowth arises which later extends out to form a coiled structure. Still later this detaches itself to form the homomorphic pair of sex-chromosomes each with a secondary terminal constriction".

The observations and interpretations made by the present writer regarding the origin and behaviour of the sex-chromosomes during the microsporogenesis of *C. indica* seem to differ very markedly from those made by Kumar and Deodikar. In this connection it has been observed that the pair of sex-chromosomes which is heteromorphic and not homomorphic, differentiates along with the other eleven autosomes and that though it has been found to remain attached to the nucleolus, no evidence has been obtained in support of Kumar and Deodikar's observation that it originates from the latter. In recent years (Gates, 1939) it has been reported that the sex-chromosome pair is nucleolar, and as such the significance of its association with the nucleolus (*cf.* also Bhaduri and Bose, 1947) as has been observed during the present investigation, becomes apparent.

#### V. SUMMARY

A study of the somatic mitosis of *Coccinia indica* has shown that there are 24 chromosomes in the nuclei of both male and female plants, of which one pair is heteromorphic (XY) in the male and homomorphic (YY) in the female.

The somatic metaphase spindle originates from the polar caps which appear as a result of the shrinkage of the prophase nucleus.

During synizesis of the pollen-mother cell the tapetal cells become binucleate. In certain cases the nuclear divisions are followed by cytokinesis. The two nuclei of the daughter cell again divide, and the later nuclear divisions may or may not be followed by wall formation.

At diakinesis of the pollen mother cell 12 bivalents have been seen, of which one is heteromorphic and has been found to be attached to the nucleolus.

A polar view of metaphase I shows 12 bivalents. One of these is decidedly bigger than the others and is the sex-chromosome pair (XY), whose identity could be followed during most of the later stages of meiosis.

Various forms of abnormalities have been noted, among which cytotoxicity and the binucleate condition of the pollen mother cells appear to be the most common.



Laggards have been found to be characteristic of the species. The lagging univalents organize micronuclei which subsequently divide along with the dyad nuclei, resulting in polyspory.

## VI. ACKNOWLEDGEMENT

In conclusion the writer takes this opportunity of expressing his thanks to Dr. I. Banerji under whose guidance this work was carried out in the Botanical Laboratory of the Calcutta University.

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# LEPTOCEPHALI OF THE GULF OF MANAAR\*

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IN view of the scanty records of Indian Leptocephali (Kaup, 1856; Southwell and Prashad, 1919; Deraniyagala, 1934; Gopinath, 1946 and Nair, 1946 and 1947), a thorough and systematic study of these larvæ together with a knowledge of their distribution is an important and necessary prelude to the study of the biology of the Indian eels of which we know so little while great advances have been made in the study of their European counterpart.

During the course of a three days' visit to Krusadai Island, Gulf of Manaar, early in July, 1947, numerous specimens of Leptocephali were collected each day from the shore seines operated by fishermen from Kutikal Point of Rameswaram Island. On the 8th July 1947, the larvæ occurred in enormous numbers composed of two varieties of *Congrellus anago* and *Uroconger lepturus*. It may be mentioned here that *Stolephorus commersonii* and *Chirocentrus dorab* formed the predominant catch of fish in the shore seines during these days. As may be expected while using such nets, all the specimens were unfortunately taken in a dead or dying condition and consequently no observations on their metamorphosis could be made.

## *Congrellus anago* (Schlegel)

6th July 1947	..	10 specimens.
7th July 1947	..	11 specimens.
8th July 1947	..	72 specimens.

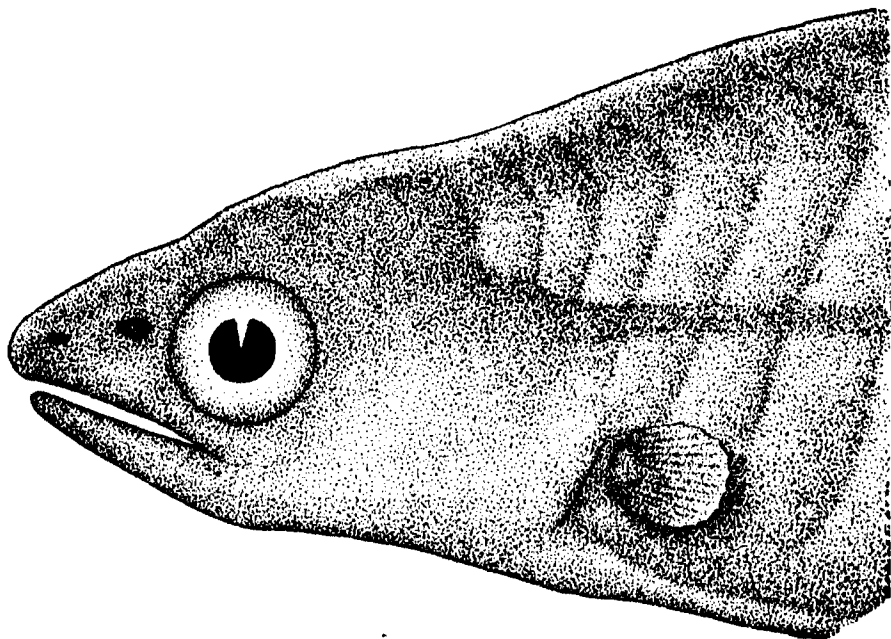
The Leptocephalus of *Congrellus anago* was recorded recently by Gopinath (1946) from the Trivandrum Coast. Apparently the identification of the larva is based on circumstantial evidence afforded by the presence of the elvers of *Congrellus anago* along with the Leptocephalus and, therefore, confirmation of Gopinath's correlation appears to be necessary.

**Measurements.**—Total length 142 mm.; length of head 4 mm.; length of trunk 110 mm.; length from anus to tip of tail 28 mm.; length from tip of snout to origin of dorsal fin 120 mm.; maximum height 13.5 mm.

The Leptocephalus is transparent, long and flattened with 116 relatively broad myotomes 1.5 mm. wide near the middle region of the body. A

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narrow transparent region to which the myotomes do not extend is present on the ventral side above the alimentary canal. The small, triangular head has a bluntly pointed snout (Fig. 1). The upper jaw is slightly longer than



TEXT-FIG. 1. Head region of the Leptocephalus of *Congrellus anago*.  $\times$  ca 18.

the lower and in all the specimens the larval teeth have fallen off. No indication of the formation of the adult set of teeth is seen in any of the larvæ. The cleft of the mouth is straight and extends posteriorly to the level of the centre of the eye. The alimentary canal is very long and straight and forms a prominent gradually broadening region between its commencement and the 24th myotome. While in a few of the specimens the anus is placed below the 82nd myotome, in the majority, however, it has shifted anteriorly, and this fact taken in conjunction with the absence of the larval set of teeth suggests that the larvæ have reached their limit of full growth and have just begun to metamorphose into the elvers. It is possible that in the fully grown larva, before the onset of metamorphosis, the anal opening may be situated under a still posterior myotome. The dorsal fin is very short with closely-set developing fin-rays while the anal is slightly longer with the rays well developed and prominent. The caudal fin is confluent with the vertical fins whose rays indistinguishably merge with those of the former.

The head is devoid of pigmentation. The presence of black stellate chromatophores at the bases of all but a few of the anteriormost rays of the anal

fin and of the caudal fin appears to be a distinguishing feature of the larva of *Congrellus anago*, and this pigmentation is clearly visible even to the naked eye. Similar chromatophores are present at the bases of the better developed posterior fin-rays of the dorsal fin. Elongated irregularly arranged black chromatophores are present along the dorsal side of the alimentary canal. In some specimens, a row of black chromatophores is found along the ventral half of the myocommas of the posterior half of the body. These groups of pigment cells become prominent towards the caudal end.

It is known that the *Leptocephalus* of *Congrellus anago* occurs in good numbers from the beginning of November till the end of February along the Trivandrum Coast (Gopinath, 1946). This larva, which appears to be the common *Leptocephalus* of the Gulf of Manaar, has not so far been recorded along the Madras Coast in the regular plankton collections made at the University Zoological Research Laboratory, Madras, during the last ten years. But we know that the recorded habitat of the adult is from the Coromandel Coast of India to Malay Archipelago (*Congromuræna anago*, Day, 1889). We are thus confronted with certain interesting problems about the distribution of the larva and the adult of *Congrellus anago*.

*Uroconger lepturus* (Richardson)

6th July 1947	..	3 specimens.
7th July 1947	..	11 specimens.
8th July 1947	..	21 specimens.

An account of the *Leptocephalus* of *Uroconger lepturus* occurring in the Madras plankton and the correlation between myotome and vertebral counts of the larva and the adult respectively has already been given (Nair, 1946).

All the larvæ in the present collection are edentulous with great reduction in the length of the alimentary canal and the height of the larva which varied from 8-9 mm. These changes indicate that the larvæ are in a more advanced stage of metamorphosis than those collected from the Madras plankton. This is corroborated by the appearance of new chromatophores in the head region consequent on the commencement of metamorphosis. Five to six black stellate chromatophores are present in a horizontal line near the heart region with one or two stray pigment cells above them. Two or three similar chromatophores occur at the middle region of the upper jaw.

The *Leptocephalus* of *Uroconger lepturus* can, therefore, now be taken as common on the East Coast of India. *Leptocephalus acuticaudatus* was collected by Kaup (1856) from Malabar and though the descriptive account of the larva is meagre and unsatisfactory, Kaup's figure of it bears a strong

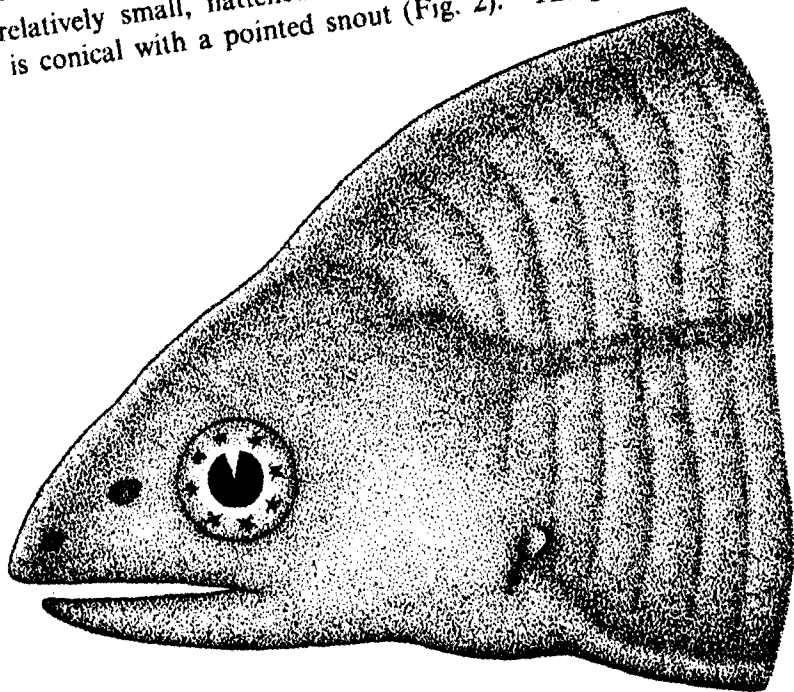
resemblance to the present larva in certain features. Therefore, it is possible that *Leptocephalus acuticaudatus* may belong to the genus *Uroconger*.

*Murana* sp.

6th July 1947 . . . 1 specimen.

*Measurements*.—Total length 66 mm.; length of head 4 mm.; length of trunk 43 mm.; length from anus to tip of tail 19 mm.; length from tip of snout to origin of dorsal fin 38 mm.; maximum height 9 mm.

The relatively small, flattened and leaf-like larva has 210 myotomes. The head is conical with a pointed snout (Fig. 2). The gape of the mouth



TEXT-FIG. 2. Head region of the *Leptocephalus* of *Murana* sp.  $\times$  ca 18.

is straight and extends to a level with the middle of the eye. The larval set of teeth has fallen off and no indication of the formation of the adult set is present. The course of the alimentary canal is straight with the anal opening below the 126th myotome. The pectoral fin is present as a very small and rudimentary structure.

The eye is tinged yellow with eight black stellate chromatophores round the pupil. The body is devoid of chromatophores except for the presence of a few minute black ones at the bases of the posterior region of the anal and the caudal fins.

The larva very closely resembles that of *Muræna macrura* described by the author (1947) from the Madras plankton; the coloration of the eye particularly is very striking, and it is probable that the larva belongs to a species of *Muræna* not unrelated to *Muræna macrura*. Deraniyagala (1934) has recorded two larvæ belonging to Murænidæ from Ceylon waters. Kaup's (1856) figure of *Leptocephalus dussumieri* which was collected from Malabar and imperfectly described by him reminds one of a Murænid larva. Many Murænid larvæ which look alike not only in appearance but also in other characters should be expected from the Indian Seas where many species are known to occur.

*Murænesox cinereus* (Forskål)

Three specimens of the Leptocephalus of *Murænesox cinereus* collected on the 3rd May 1947, by my colleague, Mr. P. R. Sadasivan Tampi, from a shore seine worked from Kutikal Point were handed over to me with the information that *Dussumieria hasseltii* formed the bulk of the catch in the net.

An account of the larva of *Murænesox cinereus* together with the changes undergone by it during metamorphosis into the adult has previously been given by the author (1947).

The Leptocephalus of *Murænesox cinereus*, like that of *Uroconger lepturus*, can also now be considered as common on the East Coast of India.

My thanks are due to Dr. H. Srinivasa Rao, Chief Research Officer, for going through the manuscript and making helpful suggestions in improving the format of the paper.

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# PRELIMINARY TRIALS IN THE PROPAGATION OF *BRACON (MICROBRACON) GREENI* ASHMEAD ON UNNATURAL HOSTS

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## INTRODUCTION

Mass propagation of parasites and predators is of great practical importance in biological control work, and their breeding for large-scale releases to a great extent depends on a continuous and adequate supply of host material. In most cases it is not possible to have a sufficiently large supply of the natural host at all times of the year, so that it becomes imperative to find out alternate hosts on which the parasites could be bred in the laboratory. There are numerous instances wherein alternate hosts have been employed successfully for an artificial rearing of the parasites although they may not have been recorded from these hosts under natural conditions, as for example, the mass breeding of the egg parasite, *Trichogramma evanescens minutum* on the eggs of various lepidopterous pests of stored products and the breeding of the braconid, *Macrocentrus ancylivorus*, a larval parasite of the Oriental fruit moth, on the potato tuber worm.

The control of the two major predator enemies of lac, *Eublemma amabilis* and *Holcocera pulvereana* on a mass scale by utilising their natural enemies has been engaging the attention of this institute since 1930. Results of the biological control experiments carried out during 1941-1944 have already been published (Negi, Gupta, Misra, Venkatraman and De, 1945) and papers on the mass breeding of *Bracon greeni* on natural and alternate (unnatural) hosts were read at the annual session of the Indian Science Congress by Gupta (1938) and Venkatraman (1945) respectively. The present paper gives an account of the studies on the olfactory responses of the parasite in relation to its mass production, together with the result of preliminary attempts in the propagation of *B. greeni* in the laboratory on some unnatural hosts (Annual Reports, 1930-45).

## HOST SELECTION BY *Bracon greeni*

*General behaviour of parasite.*—*B. greeni* Ashm., is a primary, ectophagus, larval parasite of the lac predator, *Eublemma amabilis* Moore,

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occurring in the major lac growing areas in India (Misra, Negi and Gupta, 1930). The biology and habits of the parasite have been studied in great detail by Gupta. The parasite is specific in the selection of its host and so far has not been found to parasitise any other host in nature.

The female parasite, as soon as it comes in the vicinity of a host, is attracted towards it, and this initial attraction of the parasite to the host is mainly due to the odour of the host larva. Having reached the region in which the host is located, the female parasite tries to find out the exact spot by vibrating its antennæ. The olfactory sense appears to be located in the antennal segments; adults devoid of antennæ or adults having their antennæ coated with shellac varnish, seem to evince little interest for oviposition. When once the parasite is within the reach of the host, the texture of the dome-shaped covering of the host larva, the size of the host larva and its movement while the parasite tries to sting it, probably give the final stimulus to oviposit. The parasite deposits its eggs upon or near the host after paralysing the larva with a toxic fluid while stinging. The braconid parasitises the middle and later stages of *E. amabilis* larva.

*Odour of the host and its web, the chief attracting factor.*—Preliminary studies on oviposition response of the female parasite to stimuli such as size, texture and odour of the host showed that odour emanating from the host larva and its associated spun envelope was the dominant factor in host selection. Hence the responses of the parasite to this factor alone were investigated by means of a modified McIndoo's olfactometer devised by Thorpe and Jones (1937). The results detailed in Table I to IV confirm the conclusions drawn from preliminary experiments.

Females used in these experiments were bred in the Laboratory and had not previously oviposited, but had copulated. They were mostly about 2 to 3 days old and were fed with cane sugar solution. All precautions to avoid any possible errors were taken. The experiments were carried out in a dark room, where the temperature and humidity were fairly constant. The position of the objects used for testing odour, was alternated from one arm to the other at each trial and the olfactometer was thoroughly washed after every three experiments.

#### RESULTS OF EXPERIMENTS

1. The degree of attraction exerted by smell of *Eublemma* larva was tested first. Eight medium-sized larvæ of the natural host were removed from their galleries and kept in one arm of the apparatus, the other arm being left blank. Fifty gravid females which had not oviposited previously, were used in each of these experiments. Results are presented in Table I.



TABLE I  
Response of *Bracon* (reared on *Eublemma*) to *Eublemma*  
larva in olfactometer

Sr. No. of Expts.	No. of females entered in the arm of the olfactometer			Remarks
	Containing <i>Eublemma</i>	Blank	Total	
1	10	6	16	Out of 186 choices the arm containing <i>Eublemma</i> was favoured by 134 females (72.1%) and the blank arm by 52 females (27.9%). The standard deviation calculated from the formula $Sd = \sqrt{\frac{S(x-x)^2}{N \times N - 1}} = \pm 2.28$ Results considered significant since the value 72.1% exceeds 27.9% by more than twice the standard deviation
2	17	5	22	
3	10	8	18	
4	21	12	33	
5	15	3	18	
6	18	4	22	
7	12	3	15	
8	24	11	35	
9	7	0	7	
Total	134 = 72.1%	52 = 27.9%	186	

2. Next, the intensity of attraction exerted by the odour of the web spun by *Eublemma* larva on *Bracon* reared from *Eublemma* was tested. Fresh webs of larva lined with pellets of its excreta were placed in one arm and the other was left blank. Fifty individuals were used in each of these experiments.

TABLE II  
Response of *Bracon* (reared on *Eublemma*) to the web spun by  
*Eublemma* larva, in olfactometer

Sr. No. of Expts.	No. of females entered the arm of olfactometer			Results
	Containing <i>Eublemma</i> web	Blank	Total	
1	11	7	18	Out of 291 choices, <i>Eublemma</i> web was preferred by 203 females (69.8%) as against the blank arm by 88 (30.2%). Standard deviation $Sd = \pm 2.7$ Results considered significant.
2	9	7	16	
3	20	3	23	
4	18	4	22	
5	29	11	40	
6	15	8	23	
7	7	7	14	
8	12	6	18	
9	26	11	37	
10	8	9	17	
11	31	5	36	
12	17	10	27	
Total	203 = 69.8%	88 = 30.2%	291	

3. Here the reaction of *Bracon* to a choice of odour of *Eublemma* larva and its web was studied. Eight larvæ of *Eublemma* were placed in one arm and fresh larval webs of *Eublemma* in the other. Forty-five females were used.

TABLE III

Response of *Bracon* (reared from *Eublemma*) to *Eublemma* larva and larval web, in olfactometer

Sr. No. of Expts.	No. of females entered the arm of olfactometer containing			Results
	<i>Eublemma</i> larva	<i>Eublemma</i> web	Total	
1	10	11	21	Out of 262 choices <i>Eublemma</i> larva was preferred by 121 females (46.2%) as against <i>Eublemma</i> web preferred by 141 females (53.8%). Standard deviation $Sd = \pm 1.97$  Results significant.
2	12	17	29	
3	16	10	26	
4	11	18	29	
5	14	16	30	
6	21	13	34	
7	15	16	31	
8	8	11	19	
9	5	21	26	
10	9	8	17	
Total	121 = 46.2%	141 = 53.8%	262	

4. The reaction of the third generation of *Bracon* reared on *Platyedra gossypiella* to a choice of two hosts, viz., *Platyedra* and *Eublemma* was investigated. Eight larvæ of *Eublemma* were placed in one arm and eight *Platyedra* larvæ in the other, care being taken to use larvæ of about the same size. Twenty-five gravid females reared continuously for three generations on *Platyedra* were used in each of these experiments.

TABLE IV

Responses of *Bracon* (reared on *Platyedra* for three generations) to larvæ of *Platyedra* and *Eublemma*, in olfactometer

Sr. No. of Expts.	No. of females entered in the arm of olfactometer containing			Results
	<i>Eublemma</i>	<i>Platyedra</i>	Total	
1	8	7	15	Out of 119 choices, <i>Eublemma</i> was preferred by 74 females (62.1%) <i>Platyedra</i> by 45 (37.9%) Standard deviation $Sd = \pm 1.45$  Results significant.
2	10	4	14	
3	11	10	21	
4	4	4	8	
5	13	4	17	
6	9	7	16	
7	6	2	8	
8	13	7	20	
Total	74 = 62.1%	45 = 37.9%	119	

BREEDING OF *Bracon greeni*

1. *Host material*.—The natural host, *Eublemma*, is not easily obtained in large numbers from the field, throughout the year. During the months, March to May, it is scarce in the field, because most of the eggs laid by the third generation of moths from October to February on *Baisakhi* (October to June) and *Aghani* (June to January) lac crops, do not develop due to cold and those laid by the moths of the fourth generation that survive winter are greatly affected by heat in summer months (Misra, Negi, Gupta, 1930). Attempts to rear the host on a large scale in the laboratory have not been successful so far. On account of these difficulties the breeding of parasites on unnatural hosts was undertaken.

Under Laboratory conditions, *Bracon greeni* has been successfully bred on many unnatural hosts, the criterion of success being judged by the development of parasite grubs to maturity. A list of hosts tried so far is given below, the hosts being arranged according to the classification of Simmonds (1944).

## LIST OF UNNATURAL HOSTS TRIED

*Category I. Attack without much reluctance: subsequent development normal.*

*Platyedra gossypiella* (pink boll worm of cotton), *Scirpophaga nivella* (top borer of sugarcane) and *Leucinodes orbonalis* (Brinjal fruit borer).

*Category II. Attack with reluctance: subsequent development subnormal.*

*Trachylepidia fructicassiella* (Cassia fistula pod borer), *Enarmonica perfricta* (Pongamia glabra seed borer), *Corcyra cephalonica* (rice moth), *Chilo zonellus* (maize borer), *Etiella zinckenella* (pea borer), *Plutella maculipennis* (diamond back moth), *Ephestia cautella* (*Bassia latifolia* seed borer), *Cataglyphus bicolor* (red ant larva).

*Category III. No attack: no progeny*

*Earias* spp. (*Hibiscus esculentus* fruit borer), *Earias* spp. (spotted boll worm of cotton), *Gnorimoschema operculella* (potato tuber worm), *Hypsiphyla robusta* (toon shoot borer), *Bombyx mori* (mulberry silkworm), *Emmalocera depressella* (root borer of sugarcane).

A summary of results of breeding *Bracon greeni* on unnatural hosts during 1943–1946 is presented under Table V.

2. *Breeding technique*.—The method devised by Negi and Gupta for the mass breeding of parasites on the natural host *Eublemma* cited and used by Glover and Chatterji (1936) is being followed to breed the parasites on unnatural hosts also, but the mode of presentation of host larva has been modified.

*A. Methods of offering host larvæ for parasitisation.*—Several methods were tried to offer the natural host *Eulemma* for parasitisation and from the view point of maximum parasitisation the following three methods have been found most suitable in the order of merit.

(1) Small cut pieces of lac sticks containing healthy *Eulemma* larva *in situ* (Fig. 7). This method, though gives the highest parasitisation, is not always feasible, because, while cutting the *Eulemma* infested portions of lac twigs a considerable part of healthy lac is also likely to be removed from the plants.

(2) *Eulemma* larva are collected by picking from lac-bearing trees or harvested lac and each larva placed singly in a bit of lac called 'dome' (Fig. 1). The larva spins a fine web of silk and excreta over it. Such 'domes' are termed 'lac domes' (Fig. 6).

(3) Each *Eulemma* larva placed singly in a lac bit, is covered with a thin tissue paper (bleached sulphate paper about 0.02 mm. in thickness). These 'domes' are called 'tissue domes' (Figs. 2, 3 and 4).

The cut bits of lac or 'lac domes' or 'tissue domes' containing *Eulemma* larvæ are fixed to a stick having a coat of plasticene over it and these artificially made lac sticks or pseudo-sticks are presented for parasitisation.

In the case of unnatural hosts, for obvious reasons, method (1) is not possible, hence trials were given to methods (2) and (3) and various other devices, but only method (3) described above was found promising. But it lacked sufficient attraction to the parasite and in the case of agile larvæ, such as of *Trachylepidia fructicassella*, most of the larvæ escaped from the 'tissue domes' by biting out holes in the tissue paper cover. The larvæ that came out escaped parasitisation and constructed webs inside the oviposition cage, with the result that adult parasites often got entangled in the web. Therefore, methods had to be evolved to make the host larvæ inactive and acceptable to the parasite.

*B. Artificial paralysation of host.*—The two most successful methods were (1) amputation of mouth parts without injury to head capsule, and (2) coddling, by immersing the larvæ in hot water at a particular temperature for a certain time. The former method is laborious and requires skill which may be had with practice. In the case of the latter method, the temperature of water and the duration of treatment have to be determined for each species. There was some mortality of host larvæ, which were paralysed by the above methods, but pupation was prevented in most cases.

TABLE V. Summary of breeding *B. greeni* on unnatural hosts during 1943-46

Name of host	Mode of exposing the host larva	Host larvæ exposed to action of parasite			Host parasite contact in days	Adult parasites bred					Remarks on host suitability
		No. exposed	No. parasitised	Percentage parasitism		No. of males	No. of females	Total adults	Percentage of females	Adults per host larva	
1	2	3	4	5	6	7	8	9	10	11	12
1 <i>Platyedra gossypiella</i> (pink boll worm of cotton)	In 'tissue dome'; tissue covering studded with <i>Eublemma</i> excreta; not paralysed artificially.	37,451	12,967	34.6	1-5	4,923	5,992	10,915	54.9	0.8	A suitable host to breed <i>B. eremi</i> ; the larva remains quiet inside 'domes'. It can be stored in infected cotton seeds for some time.
2 <i>Trachyleptidia fructuicollis</i> ( <i>Casnia ficulna</i> pod borer)	In 'tissue domes'; covering pasted with <i>Eublemma</i> excreta; larva quietened by coddling or amputation of mouth parts.	6,526	893	13.6	1-5	213	362	575	62.9	0.6	A suitable host if available in large numbers. Requires artificial paralysation to stay in 'tissue domes'. Oviposition technique requires improvement.
3 <i>Enarmonia perfrita</i> (early seed borer of <i>Pongamia glabra</i> )	In 'tissue domes', tissue covering containing <i>Eublemma</i> excreta; coddled before presentation.	2,400	938	39.1	1-4	74	150	224	67.0	0.2	The parasite grubs do not seem to relish the juice of caterpillars, which have a repugnant odour. Attempts are being made to breed this host on edible pods to change the odour.
4 <i>Stirpophaga nitella</i> (top borer of sugarcane)	In 'tissue domes', tissue paper covering with <i>Eublemma</i> excreta; not paralysed artificially.	97	29	29.9	1-4	19	31	50	62.0	1.7	Larva remains quiet inside 'domes' and fall grown larva can subsist as many as 6 parasite grubs. Larva from second instar onwards suitable for breeding the parasite. It would be an useful substitute host, if sufficient and continuous supply could be ensured.

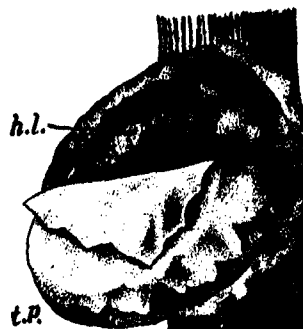
5 <i>Coryna cephalonica</i> (the rice moth)	In 'tissue dome': tissue covering studded with <i>Exblennia</i> excreta; coddled (47° C. for 6 minutes) before presentation.	474	88	16-8	1-4	20	43	63	68-2	0-8	Larva very active and has many small hairs which seem to be disliked by braconid; requires artificial paralysis to stay in 'dome'. Easy to breed in the laboratory in abundance. It will serve as a useful laboratory host, but the incidence of the pest is very low in and around Ranchi.
6 <i>Lecisnoda ortho-</i> <i>nalis</i> (brinjal fruit borer)	In 'tissue dome': tissue covering pasted with <i>Exblennia</i> excreta.	29	6	20-9	1-4	1	2	3	66-6	0-5	Larva thick skinned and active requires artificial paralysis. Further investigation is considered necessary.
7 <i>Chilo zonellus</i> (maize borer)	In 'tissue dome': tissue paper covering pasted with 'Exblennia' excreta coddled (46° C. for 5 min.) before exposure.	77	6	7-7	2-4	1	1	2	50-0	0-3	Heavy mortality of host larvæ inside oviposition cage, hence not suitable for mass breeding.
8 <i>Cataglyphis bicolor</i> (ant larva)	In 'tissue dome'; no artificial paralysis necessary.	1,006	17	1-7	2-5	1	10	11	90-9	0-6	



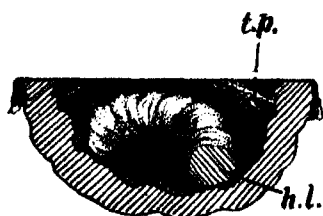
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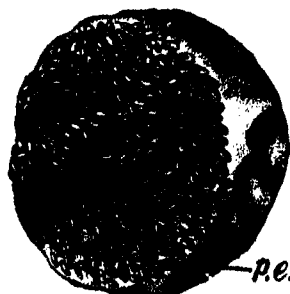
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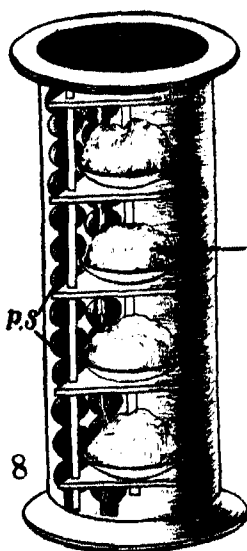
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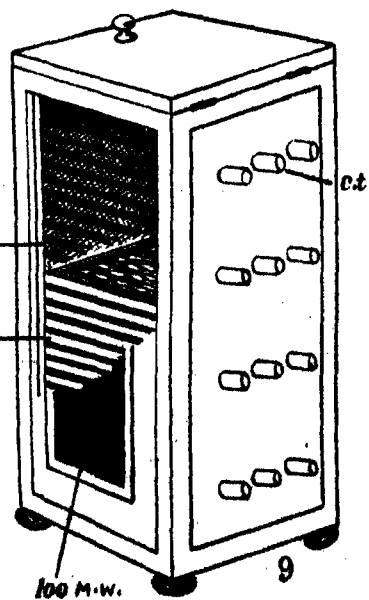


8

15 M.W.

C.S.

S.t.



9

FIGS. 1-9

(All figures diagrammatic)

FIGS. 1-9.—Fig. 1. 'Dome' of lac, enlarged. Fig. 2. 'Tissue dome,' enlarged. Fig. 3. 'Tissue dome,' partially opened to show host larva, enlarged. Fig. 4. Transverse section of 'tissue dome,' enlarged. Fig. 5. 'Tissue dome' with *Eublemma* excreta pasted over tissue covering, enlarged. Fig. 6. 'Lac dome,' naturally spun by *Eublemma* larva, enlarged. Fig. 7. *Eublemma* spun dome, *in situ*, on lac stick, enlarged. Fig. 8. Oviposition cage. Fig. 9. Emergence cage.

- |  |   |
|--|---|
| c. s. cotton swabs soaked in sugar solution. | p. s. 'psuedosticks' bearing 'domes'.       |
| c. t. collecting tubes.                      | s. t. storage trays.                        |
| h. e. hollow excavation in 'dome'            | t. p. tissue paper covering.                |
| h. l. host larva.                            | 100 m.w. 100-mesh wire-gauze on outer wall. |
| p. e. pellets of <i>Eublemma</i> excreta.    | 15 m.w. 15-mesh wire-gauze on inner wall.   |

(a) *Rendering host larva suitable for acceptance*.—The next attempt in the method of presentation was to make the tissue paper covering of 'domes' simulate the natural web of *Eublemma* in regard to odour and texture. It has been stated earlier that the odour of the natural host, *Eublemma* and its web and excreta is a dominant factor in the initial attraction of the female parasite to the host, wherefore, fresh pellets of excreta of *Eublemma* larva were pasted one layer thick to the tissue covering of 'domes' (Fig. 5). Tissue 'domes' containing unnatural hosts with and without *Eublemma* excreta were introduced in a series of identical oviposition cages, and out of 1,910 *P. gossypiella* larvæ offered in tissue domes without *Eublemma* excreta, 18.8% were parasitised and out of 2,560 *P. gossypiella* larvæ offered in tissue domes covered with *Eublemma* excreta 39.3% were parasitised. The results obtained clearly show that better oviposition results are obtained by pasting *Eublemma* excreta over this tissue covering of the 'domes'. This conclusion is further confirmed by the experiments conducted on 14,931 *E. amabilis* larvæ (natural host) offered in tissue domes without *Eublemma* excreta over them and 37,451 *P. gossypiella* larvæ (unnatural host) offered in 'tissue domes' pasted with *Eublemma* excreta; the percentage of parasitisation in the former was 29.6 and in the latter 34.6 i.e., 5% more.

It is a little tedious to collect large quantities of *Eublemma* excreta; consequently, a mixture of pellets of *Eublemma* excreta and finely powdered stick lac instead of pure *Eublemma* excreta was experimented on the 'tissue domes' and more satisfactory results were obtained than with finely powdered stick lac alone.

(b) *Oviposition cage*.—Cylindrical glass jars, 12" × 4½" with 80-mesh wire-gauze covers (Fig. 8) serve as very handy and suitable breeding cages (Gupta, 1938). Into each cage about 30 to 40 adult females and about half the number of males are introduced. 3% to 5% sugar solution is pro-



vided in the cage as food for adults. About fifty to sixty 'domes' containing host larvæ are introduced in each cage. The cages are kept in a well illuminated place and the 'tissue domes' were removed from them to storing cages, 1 to 5 days after introduction, depending on the season.

(c) *Storing of parasitised host larvæ*.—The 'domes' from the oviposition cage are stored in the emergence cage in trays (Fig. 9). The emergence cage consists of a double-walled box, 10" × 12" × 16½". The two outer side walls are fitted with 100-mesh wire-gauze screened by black cloth which can be removed or put on at will, the front wall has round holes to fix specimen collecting tubes, and the back side has a door to push the trays in. The inner three walls of the cage are of 15-mesh wire-gauze which permits the emerging braconids to pass through easily and prevents the unparasitised host, if any, from emerging out. The space between the inner and the outer walls is 2" on three sides and 1" on the back; this allows sufficient ventilation to the developing parasites. Each cage has 15 trays and each tray can hold 100 to 150 'domes'. The trays and outer walls of the cage on the inner sides are painted dull black, while the whole cage is painted white on the outside. The emerging parasites collect themselves in the glass tubes fitted in the front side of the cage, where they mate and are periodically collected.

#### DISCUSSION

Flanders (1944) observed that 'there is little doubt that odour may be critical factor in the mass production of certain species of parasitic hymenoptera, and by the manipulation of odours the reproduction of such insects may be either stimulated or repressed.' The experiments conducted to study the responses of *Bracon greeni* to the odour of the host, clearly indicate, that the smell of *Eublemma* larva and its associated spun web is an important factor in the acceptability of the host by the parasite. Of the odours emanating from the larva and the web containing excreta, the smell of the latter appears to offer more attraction to the ovipositing female.

It was also found that at least for 3 generations, rearing on unnatural hosts did not seem to result in an actual preference for the new host over the natural one. These observations are in conformity with the results of Thorpe and Jones (1937). Much has been written on this aspect of host selection by parasitic species and recently Simmonds (1944) has discussed this problem in detail and has concluded that "there is no apparent segregation of a strain particularly adapted to the unnatural host, and it would appear that the possibilities of such breeding interfering with the success of the introduction of a parasite species is remote."

The method of breeding insect parasites on unnatural hosts constitutes an important problem in biological control work. The successful breeding

of a parasite in the laboratory depends mainly upon a close study of its habits and behaviour, especially its responses to oviposition stimuli, which in most cases are found to be highly selective. In the case of *Bracon greeni*, although odour appears to play an important part in the acceptability of the host, there seems to be several other factors which operate not singly, but in combination. The main characteristics of hosts acceptable to *B. greeni* are that, (1) they must be sluggish, smooth and thin skinned, with very few bristly hairs, (2) they must be at least many times the size of the parasite, and (3) they must be surrounded by a thin rigid covering of a particular texture, with a little space between the body of the host and the covering.

#### ACKNOWLEDGMENTS

Our thanks are due to Mr. J. C. M. Gardner, Forest Entomologist, Dehra Dun, for offering helpful criticisms; to Mr. K. C. Chatterji of the department for his assistance in the breeding work and to Mr. E. Heber, Artist, for preparing the illustrations. Our appreciations are due to Dr. P. K. Bose, the Director of the Institute, for his interest in the work.

#### SUMMARY

1. This paper presents an account of the olfactory responses of the parasite, *Bracon (Microbracon) greeni* in relation to its mass production, together with the results of preliminary attempts in the breeding of the parasite on some unnatural hosts. *B. greeni* is a primary ectophagous larval parasite of the lac predator, *Eublemma amabilis* Moore.

2. With a view to finding out a suitable laboratory host for the mass breeding of the parasite, the nature of oviposition response of the female parasite produced by such stimuli as size, texture and odour of the host larva was studied. Experiments conducted with an improved McIndoo's olfactometer, indicate that the smell of the natural host (*Eublemma*) and its associated spun web plays an important part in the initial attraction of the parasite. Of the odours emanating from larva and the spun web, the smell of the latter appears to offer more attraction to the ovipositing female. It was also observed that rearing on unnatural hosts did not seem to result in an actual preference for the new host over the natural one.

3. The technique in the breeding of *B. greeni* on unnatural hosts is described in some detail. Host larvæ are exposed to the action of the parasite in 'tissue domes' (lac bits covered with tissue paper), the tissue paper covering being studded with pellets of *Eublemma* larval excreta. These tissue covering pasted with excreta simulated the natural web of *Eublemma* and offered sufficient attraction to the ovipositing female. A mixture of

pellets of excreta and finely powdered stick lac also gave satisfactory results. The larvæ of active unnatural hosts invariably escaped out of 'tissue domes' in which they were presented for parasitisation, by biting out the tissue paper covering. Of the methods tried for making the larvæ inactive, the most successful were (1) amputation of mouth parts without injury to head capsule, and (2) coddling, by immersing the larva in hot water at a particular temperature for a certain time.

4. Although *B. greeni* is a specific parasite of *E. amabilis*, it has been bred successfully in the laboratory on a number of unnatural hosts, most of them being pests of economic importance. A list of unnatural hosts tried is given. Results of breeding on unnatural hosts are presented.

Of the hosts tried in the laboratory, *P. gossypiella* (pink boll worm of cotton) appears to be the most suitable host. *S. nivella* (sugarcane top borer) will serve as an additional substitute host if a regular supply of this host material could be ensured. *T. fructicassella* (*Cassia* pod borer) and *C. cephalonica* (rice moth) also seem promising.

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## A NOTE ON SOME ABNORMAL FLOWERS OF *HIBISCUS ESCULENTUS* L.

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(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

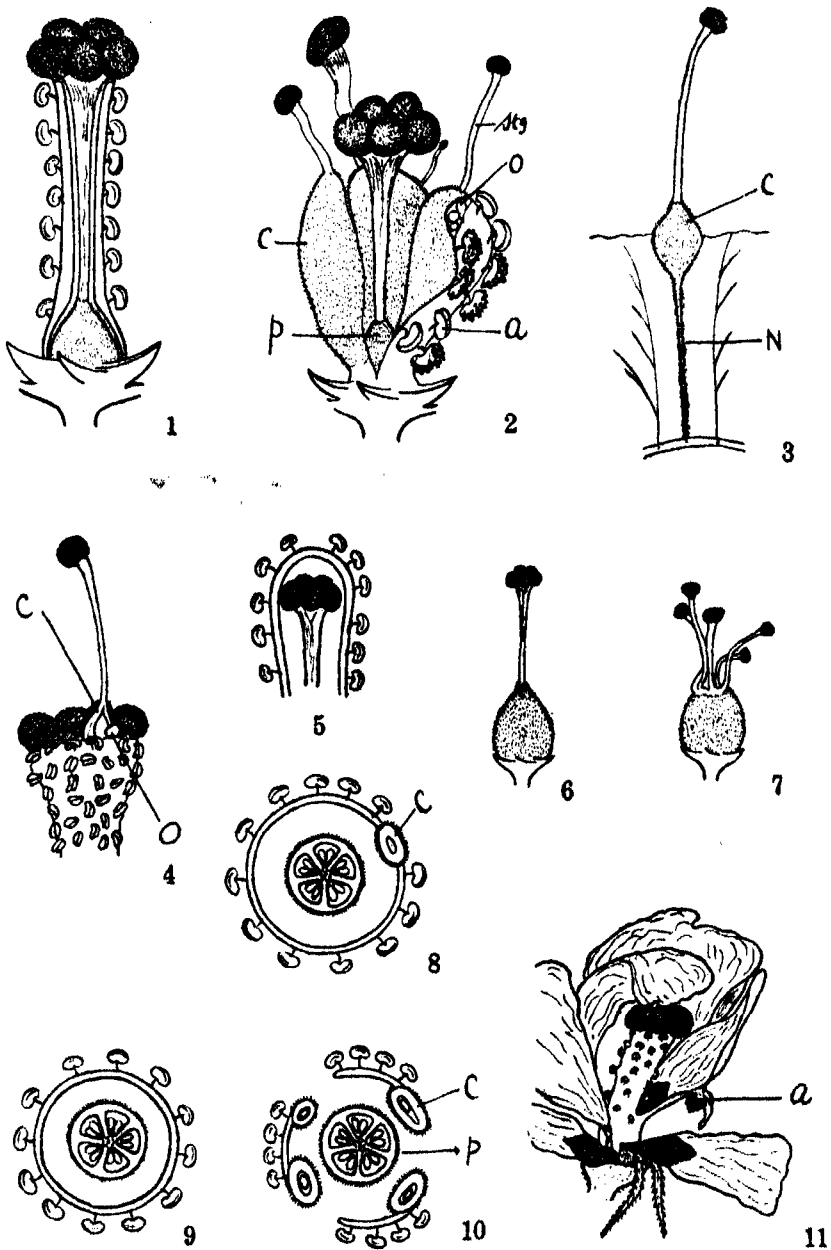
SOME interesting examples of abnormalities in the floral structures in plants of several natural orders have been recorded from time to time. Many such cases have been recorded in some species of *Gossypium* and *Hibiscus*; but, as far as the author is aware, only very few instances of such abnormalities in the flowers of *Hibiscus esculentus* have so far been reported upon.

The occurrence of intracarpellary fruits has been recorded by Delavaud (1858) in *Hibiscus tiliaceus*, and by Harris (1913) in *Hibiscus esculentus*. Such an instance has also been reported by Bergman (1932) in some hybrid forms of *Hibiscus*. The presence of extra-carpellary outgrowths has also been recorded in *Hibiscus esculentus*. Saksena (1932) found a few outgrowths from the syncarpous fruits of this species, and Venkataramani (1945) observed a second rudimentary ovary with a separate style and stigma of its own in a flower of the same species.

Wilcox and Holt (1913) and Bergman (1932) have recorded another type of abnormality in some hybrid *Hibiscus* in which a second flower was produced through an extension of the central axis of the primary flower.

Instances of petalody have been recorded in some species of *Gossypium* and *Hibiscus* (Sankaran, 1931; Ramanatha Ayyar and Sankaran, 1934; and Singh, 1935). The production of stalked stigma-like structures from the staminal tube of the flower has been recorded in *Hibiscus esculentus* by Venkataramani (1945). In this abnormal flower from the free end of the staminal tube were produced two thread-like structures resembling the style, each ending in a stigma-like body. The same flower showed another abnormality, viz., the doubling of the epicalyx—there were two whorls of epicalyx one over the other.

The occurrence of carpelody in *Hibiscus esculentus* has been recorded by Agharkar (1927). In all the cases of carpelody observed by him one to three of the topmost stamens of the staminal tube were modified into carpellary structures. According to him, the basal part of the filament became



Text-Figs. 1-11.—Diagrammatic sketches showing the abnormalities in some flowers of *Hibiscus esculentus* L.

Fig. 1. Showing a section of the staminal tube of a normal flower and the pistil.  
 Fig. 2. Staminal tube modified into three portions with carpel-like structures, a, anther;

*o.* ovules; *stg.* style and stigma; *p.* normal pistil in the middle of the three staminal portions; *c.* carpel-like structure. Fig. 3. A portion of the staminal tube with a stamen modified into a carpel-like structure (viewed from inside the staminal tube). *C.* broadened carpellary portion on the staminal tube; *N.* narrow, slender portion of the carpel with hairs extending to the base on the inner surface of the staminal tube. Fig. 4. A part of the staminal tube with a number of stamens on the outer surface and the modified carpellary structure. *C.* carpel; *O.* exposed ovule. Fig. 5. Staminal tube completely covering the stigmatic lobes. Fig. 6. A normal pistil with a single stylar column and five separate stigmatic lobes at the top. Fig. 7. An abnormal pistil with five separate styles, each ending with a separate stigma. Fig. 8. Diagrammatic sketch of the staminal tube in section showing a single stamen modified into a carpel (*C*). The normal ovary is in the centre. Fig. 9. Diagrammatic sketch of the staminal tube and ovary of a normal flower. Fig. 10. Diagrammatic sketch of the staminal tube and ovary of an abnormal flower showing the staminal tube modified into three portions. *C.* carpel-like structure; *p.* normal ovary. Fig. 11. Flower showing petalody. Note the position of the petaloid structure. *a.* anther lobe showing a few pollen grains.

broader and bore one or two ovules on the margins, thus resembling an open carpel; the middle part of the filament developed into the style while the anther was replaced by the bushy stigma.

The writer came across five types of abnormal flowers in *Hibiscus esculentus* during the years 1946-47 in the experimental plots of the University Botany Laboratory, Madras. As these types of abnormal flowers do not appear to have been recorded previously they are briefly described below in this communication.

1. *The complete covering of the stigmatic lobes by the staminal tube.*—This was observed in a flower (variety, "Podugu") which had been bagged for self-pollination. The flower was found shed, and on examination it was found that the stigmatic lobes were completely enclosed within the staminal tube (Plate III, Fig. 9; Text-Fig. 5) and were, therefore, prevented from being pollinated. The shedding of the flower was evidently due to the failure of pollination.

2. *Separation of the styles.*—This type of abnormality was met with in a flower of another variety, "American Long Green". There were five separate styles, each with a stigma of its own and all the five styles arising from the top of the ovary, unlike in the normal flower where there is a single stylar column which divides only at the top into five or more separate stigmatic lobes. In this abnormal flower four of the stigmatic lobes were observed to come out tearing through the staminal tube (Plate III, Figs. 7 and 8; Text-Figs. 6 and 7).

3. *Petalody.*—Two instances of petalody were observed by the author in the flowers of *Hibiscus esculentus*. In both the cases one of the stamens was modified into a petaloid structure (Plate III, Figs. 1 and 3). In one of

these two cases an anther lobe with apparently normal pollen grains was found on the margin of this modified structure (Plate III, Fig. 2; Text-Fig. 11). In the second flower, however, no such anther lobe was found.

4. *An instance of Carpellody*.—An interesting case of carpellody was observed in a flower of the variety P-15 originally obtained from the Punjab. In this abnormal flower the staminal tube was split lengthwise into three portions (Plate III, Figs. 5 and 6; Text-Fig. 2). In two of these three portions one of the stamens was modified into a carpel-like structure, while in the third two stamens were similarly modified into carpellary structures (Text-Fig. 10). In each of the two former portions the modified carpel was broad and elongated and was attached to the staminal tube portion on one side. The staminal tube portion was normal with the usual large number of stamens. The new carpel-like structure had a long style ending in a large and round stigma. The upper portion of the carpel was slightly open exposing some of the ovules. The outer surface of the carpellary portion was covered with hairs as in the normal carpel of the flower.

In the third portion of the divided staminal tube two stamens were modified into carpel-like structures and between these two was seen the attached staminal tube portion (Text-Fig. 10). One of the carpels on the staminal tube portion was large and similar to the carpel of the other two portions of the staminal tube with the upper portion slightly open exposing some of the ovules. The other carpel was smaller and had a very short style with a small stigma at the top. This carpel was not open but was completely closed.

This abnormal flower differed from the normal one in that the staminal tube was modified to contain within the normal style and five stimagtic lobes at the top (Plate III, Fig. 6; Text-Fig. 2).

5. *A second instance of Carpellody*.—A second instance of carpellody was observed in a flower of another variety, "P&l". In this case one of the stamens of the staminal tube was modified into a carpel-like structure. The staminal tube was complete and otherwise quite normal (Plate III, Fig. 4; Text-Fig. 4). When the staminal tube was split open the small modified carpellary structure was seen slightly broadened at the top of the staminal tube with the lower portion of the carpel very narrow and continued downward on the inner face of the staminal tube (Text-Fig. 3). It had a long style terminated by a capitate stigma. The surface of the carpel and its narrow lower portion were covered with hairs as in the normal carpel of the flower. The broadened upper portion of the carpellary structure was open

towards the outside above the level of the staminal tube exposing a single ovule (Plate III, Fig. 4; Text-Fig. 4).

This modification resembles to a certain extent the one recorded by Agharkar (1927). But in the case of Agharkar's specimens one to three stamens were modified into carpels, whereas in the present case only a single stamen was modified into a carpellary structure.

It may be interesting to note that all these abnormal flowers were observed on old plants at the end of the growing season. Agharkar (1927) has also mentioned that the flowers showing carpellody observed by him were almost always borne on old plants.

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## EXPLANATION TO PLATE III

- FIG. 1.\* Flower showing petalody.
- FIG. 2. An enlarged view of Fig. 1. Note the position of the petaloid structure and the anther lobe with some pollen grains (A).
- FIG. 3. Another flower showing petalody. Note the position of the petaloid structure on the staminal column.
- FIG. 4. A portion of the staminal tube with one of the stamens modified into a carpel-like structure. Note the open carpel with a single exposed ovule (o).
- FIG. 5. An abnormal flower showing the staminal tube modified into three portions.
- FIG. 6. Another view of Fig. 5 showing the modified staminal portions and the normal pistil in the middle of the three staminal portions.
- FIG. 7. The abnormal flower showing four stigmatic lobes coming out tearing through the staminal tube.
- FIG. 8. A normal pistil, and the abnormal pistil with five separate styles each with a stigma of its own.
- FIG. 9. Flower showing the staminal tube completely covering the stigmatic lobes.



1



2



3



4



5



6



7



8



9



# NUTRITION IN THE ADVANCED EMBRYOS OF THE SCORPION: *PALAMNAEUS SCABER* THORELL.

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DURING my study of the development of the scorpion *Palamnaeus scaber* Thorell, I came across a peculiar feeding mechanism in the later embryos. Unlike the heavily yolked eggs which are characteristic of most scorpions, the Scorpioninæ to which *Palamnaeus* belongs have yolk-free eggs and from the earliest period of development the embryos in these are nourished from maternal tissues. This aspect has been studied by Laurie (1891), Poljansky (1903), Pavlovsky (1924) and Pflugfelder (1930), but these works have been confined more to the earlier stages.

In the later stages in the embryonic development of *Palamnaeus* when the bulk of the embryo has considerably increased and nutritional demands are great, a highly specialised type of feeding apparatus is perfected, of which I give below a brief account.

The developing embryo of *Palamnaeus* lies in a diverticulum of the ovarian tube (Fig. 1). When the egg is fertilised and begins to segment, this diverticulum is only 1.7 mm. long, but soon it undergoes remarkable changes. It rapidly elongates, enlarges and its apex, beyond the position of the embryo, grows rapidly into an elongated process, which has been named by Laurie (1891) the "appendix" (Fig. 1, *apx.*). The following measurements would serve to indicate the rapid enlargement of the diverticulum and its appendix.

Stage of embryo	Length of Diverticulum	Length of Appendix	Total length
	mm.	mm.	mm.
Fertilised egg beginning to segment	1.7	..	1.7
4 blastomere stage ..	3	4.5	7.5
Segmentation completed ..	4	9	13

An advanced embryo in relation to its diverticulum and appendix is shown in Fig. 2. The embryo is 12 to 13 mm. long, has all the parts fully formed and, except for some details, is a little scorpion lying compactly

packed, in the highly stretched diverticulum. No vital connection of the embryo with the maternal follicular wall is noticeable.

The "appendix" lies free in the hæmolymph. Its distal end which may be called the *end piece* (*ep.* Figs. 3 and 4*A*)—"endknopchen" of Pflugfelder (1930)—consists of a mass of clear cells which take up vital stains readily (Fig. 5*B*). It forms an absorbing organ. Its cells absorb nutriment

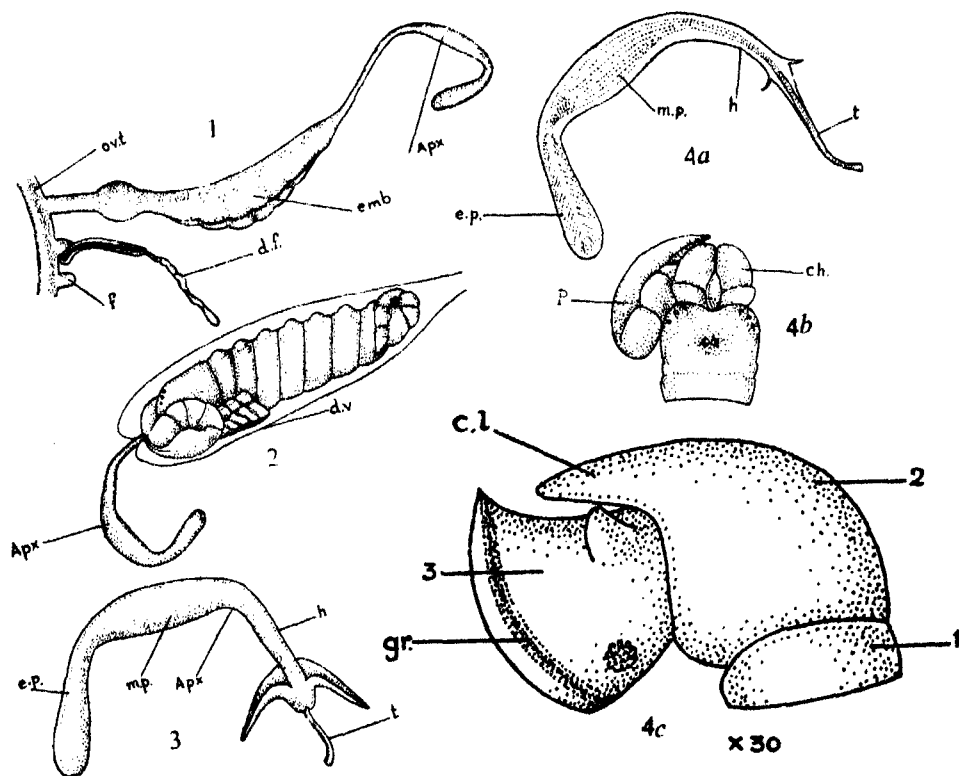


FIG. 1. Developing embryo of *P. scaber* in its diverticulum  $\times 12$ . *apx.* appendix; *d.f.* degenerating follicle; *emb.* embryo; *f.* developing ovarian follicle; *ovt.* ovarian tubule.

FIG. 2. An advanced embryo of *P. scaber* in its diverticulum (*dv.*). The left wall of the diverticulum is removed so as to expose the embryo *in situ* holding the proximal end of the appendix (*apx.*) in its mouth  $\times 7$ .

FIG. 3. An appendix removed from the diverticulum of an advanced stage. The proximal end of the appendix (*apx.*) forms the teat (*t.*)  $\times 15$ .

FIG. 4*a*. A schematic diagram of the appendix. *ep.* end piece; *h.* handle; *mp.* the swollen middle region; *t.* the "teat". Fig. 4*b*. Dorsal view of the anterior end of an advanced embryo.  $\times 9$ . *ch.* chelicera; *P.* pedipalp. Fig. 4*c*. A chelicera of an advanced embryo seen from the inner side.  $\times 30$ . 1, 2, 3, the segments of the chelicera; *cl.* the claw of the second segment; *gr.* the chitinous groove on the inner side of the third chelical segment.

materials from the ~~hemolymph~~ lymph. The cells of the middle region however are arranged to form a clearly marked out longitudinal core which extend through the middle of the next region also—the ‘appendixkörper’ of Pflugfelder. This region which may be called the *middle piece* (m p Figs 3 and 4 A) has the core thicker than the other parts. The cells making up this central core in the appendix are seen to have undergone a special development. In the earlier stages they form a solid rod of cells but in further development their cell walls become considerably thickened and their protoplasmic contents degenerate so as to ultimately form a system of conducting canals. In transverse sections these present an appearance strongly resembling that of plant tissues with their conducting vessels (Figs 5B c c)

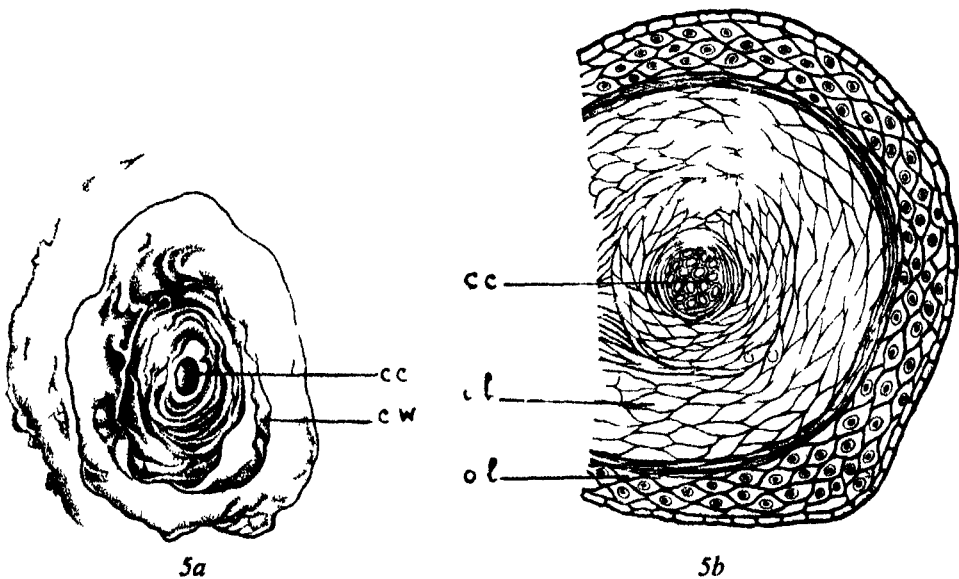


Fig 5 a Transverse section of the chitinated proximal part of the appendix forming the 'teat'  $\times 350$  cc central canal (in some two or even three canals are not ced) cw chitinated wall Fig 5 b T S of the distal end of the appendix—the end piece  $\times 200$  il inner cellular layer ol outer cellular layer cc central canals forming the core

Thus extending from the ‘end piece’ through the core of the ‘middle piece’ is a system of tiny canals or lacunæ. This lacunæ bearing core is found to be specially thick in the middle piece which has a swollen appearance and evidently serves as a reservoir for the absorbed fluids.

The proximal part of the appendix as seen externally is narrow and has been termed ‘steil’ (Pflugfelder, 1930) or *handle* (Fig 3 h). The structure of this is essentially the same as the foregoing, differing only in that the central canals are few so that the core here is very narrow. Corresponding

to this the external diameter of this region also is very small. Evidently, the numerous lacunæ of the "middle piece" have converged into these few tubes of this region. Towards the proximal extremity the central core of this region shows a gradual chitinisation of the walls of the tubes.

Beyond this part, the central core of the "appendix" which has already been noticed to begin to get chitinised, continues inside the diverticulum, as a curved chitinised tube, into the hollow of which the several canals of the middle region of the appendix lead (Fig. 5 A). This chitinised tube (Fig. 3, *t*) is inserted at the mouth of the fœtus and it is through this tube that the nutriment, absorbed by the appendix, is passed into the mouth of the embryo and so this part may be termed a "teat" (Fig. 3, *t*). Examination of living embryos showed that the pharynx with its radial muscles well developed and the gut too, exhibited regular pulsating movements indicating that definite suction is applied by the embryo to the tip of the "teat" of the appendix and that nutriment is definitely sucked in—not merely absorbed by osmosis. This part of the mechanism which I have called the "teat" is not mentioned by Pflugfelder; either he has missed it or it does not occur in *Hormurus australasiae* on which he made his observations. Fig. 4 A is a schematic diagram of the appendix as in *Palamnæus scaber*.

The "teat" described above is held in position by the chelicerae of the embryo which are developed very early and show special structural adaptations for this purpose (Figs. 4 *b* and 4 *c*).

The chelicerae formed long before the other appendages are even indicated. When their full embryonic development is reached they project in front of the prosoma and have a curious form differing widely from the adult structure. The first segment is short. The second is longer and is twisted so as to be directed downwards. Anteriorly it is produced into a finger-shaped claw. This claw is, in the adult, placed on the inner side of the appendage but here it is anterior in position. The third segment, which in the adult is outer and forms the movable claw is placed here just behind the anterior claw and has a very characteristic form. Though it is movably articulated it is not claw-like and is not opposed to the anterior claw to form a chela as in the adult. Here, it is a broad, shield-like structure, flat on its inner aspect and convex on the outer. The flat inner aspect is in contact with the corresponding structure on the opposite appendage. Thus the third segment of the two chelicerae are directed to the median line and meet by their inner, flat surfaces. Between these the chitinous tube or 'teat' which forms the proximal part of the appendix is held firmly in position. There are chitinous grooves on these opposing surfaces in which the teat lies (Fig. 4 *c*).

During gestation the liver of the scorpion is seen to undergo certain changes. It is comparatively large and occupies the greater part of the body space during the earlier stages of gestation. Estimates of their net weight showed that the liver in these early stages has on an average 15% of the weight of the body. As the embryos develop the liver loses weight rapidly and dwindles in size appreciably. By the time the embryos are fully developed and ready to be extruded, the liver is highly emaciated and represents only on an average 7% of the weight of the body. In such estimates there are considerable chances of error as a recent feed would alter the size and weight of the liver. But in the above estimate care has been taken to avoid such errors as far as possible by taking specimens under more or less similar conditions. This would indicate that the reserve food materials stored in the liver are taken up by the blood and from it the appendices of the ovarian diverticula rapidly absorb them and pass them on to the developing embryos.

A special feature with regard to this feeding mechanism is that it is entirely of maternal origin, the fœtus contributing no part to it. By the time that the zygote begins to segment, this nutritional structure also begins to develop. The only modification in the embryo in connection with this feeding mechanism is an adaptation of its chelicerae, as mentioned above, for holding the terminal duct or "teat" in position. Laurie (1891) who noticed the appendix of the ovarian diverticulum, correctly recognised it as a feeding mechanism but believed that its "solid core" is being gradually chewed and digested by the fœtus. In the early embryos where this mechanism is not perfected, in place of the chitinous teat-like tube there is a solid rod of chitin-forming cells. This structure as seen in the earlier stages might have suggested this explanation to him.

In typical 'placental' nutrition of fœtuses, capillaries from specialised maternal tissues get into intimate relation with capillaries from the fœtus and thus nutriment from the maternal blood passes into the fœtal blood. In some viviparous fishes there are "appendicula" which absorb nutriment from the uterine fluid but this is not passed into the mouth. In some Elasmobranchs, however, Ranzi (1934) has shown that the uterine secretion is absorbed into the gut through the mouth or spiracles. But even this is only sucking in of the fluids around and a special teat-mechanism is absent. In the peculiar male of the angler fish (*Ceratias holboelli*) which lives as a 'parasite' on the female, a vascular process from the latter fits into the mouth of the former (Regan, 1930). This connection, however, becomes highly vascular and the real passage of nutriment is from the blood of the female to the blood of the male.



Among the viviparous Arthropods there are no instances to be compared with this feeding arrangement. In *Hemimerus* (Heymons, 1912) the growing embryo is nourished by a secretion but this is merely absorbed by the embryo. In *Hesperoctenes* (Hagan, 1931) also, a secretion of the ovarian wall nourishes the embryo but this is absorbed by the "pleuropodial membrane" formed by the growth and fusion of the pleuropodia. In *Glossina* (Roubaud, 1909) there is a milk-secreting gland which opens into the uterus in which the foetus lies. But nowhere do we have a nutrient fluid-secreting mechanism with a definite teat inserted into the mouth of the foetus by which the secreted nutrient matter can be directly passed into the alimentary canal of the latter. Finally a remote parallel may be suggested with the condition in a marsupial. But here it is the newly born young that are fed at the maternal teat which is external whereas in the scorpion it is the intrafollicular foetus that is fed at an internal teat.

#### FATE OF THE FEEDING MECHANISM AFTER THE BIRTH OF THE YOUNG

The embryos thus richly nourished rapidly attain maturity and are ready to be born. Each now gets detached from the teat and push backwards out of the diverticulum into the ovarian tube. The wall of the ovarian tube is at this time highly elastic and gets distended with the young ones. Their backward propulsion is continued through the ovarian tubules and ultimately one by one they come out.

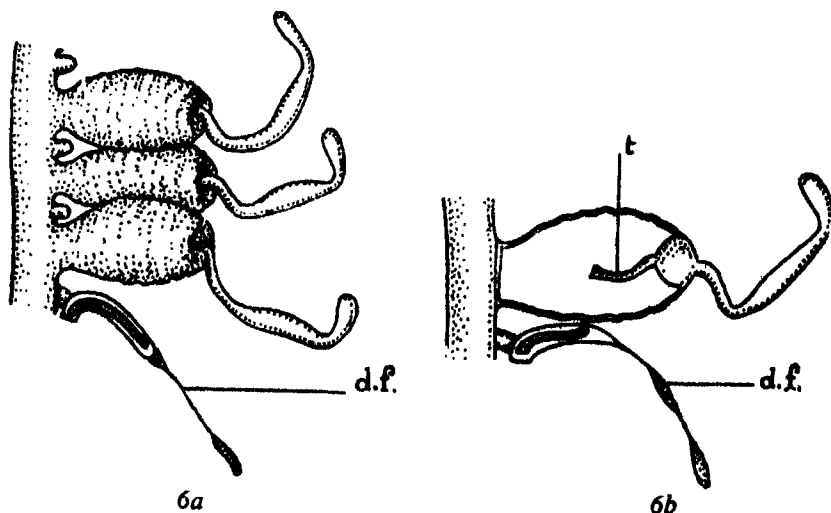


FIG. 6a. Part of an ovarian tube from which the young scorpions have been recently extruded.  $\times 7$ . d.f., degenerated diverticulum. Fig. 6b. Same as 6a but the wall of the diverticulum opened to show the "teat" (t) inside  $\times 7$ .

After the young have passed out of the diverticulum the latter undergoes remarkable changes (Figs. 6 *a* and 6 *b*). The feeding mechanism is left in the same condition as when it was functioning for the embryo. The teat, now released from the embryo, lies free in the diverticulum which has shrunk considerably and is filled with a fluid. The whole structure soon gets cut off from the wall of the ovarian tube and rapidly undergoes degeneration. A few stages in the breakdown of the diverticulum and appendix are shown in Figs. 6 *a* and 6 *b*. The last part to break down naturally is the chitinous teat, which is seen to persist even after the rest of the diverticulum has disappeared (Fig. 6 *a*).

The newly born young has the peculiar nature of the foetal chelicera still retained: in this stage it does not and cannot feed but remains mounted on the mother's back waiting for the first moult. At this event, which takes place in a few days, the shield-like third joint is cast off and in its place an opposable claw as in the adult is formed which makes the chelicera a typical chelate structure. After this only, the young one shifts for itself and feeds.

#### SUMMARY

A highly specialised embryonal nutritional apparatus seen in the embryos of *Palamnaeus scaber* Thorell. is described. The eggs are yolkless and their entire development takes place in diverticula of the maternal ovarian tubes. During development each embryo enlarges enormously in bulk and so naturally has to be richly nourished. For this purpose the distal end of the diverticulum is drawn out into an "appendix" which forms an absorbing organ and lies free in the hæmolymph from which nutrient materials are taken up. The middle portion of the appendix appears to serve as a reservoir while its proximal end is a hollow chitinised tube—"the teat"—inserted into the mouth of the embryo. The absorbed nutrient fluids pass into the gut of the embryo through this "teat". This mechanism thus suggests the arrangement of a "feeding-bottle".

Some leading types of relationship between viviparous animals and their embryos are recounted to show that there is nothing parallel to this mechanism in any of the well-known examples. At birth the embryos alone come out, the diverticula with the feeding mechanisms being retained in the maternal hæmocoele, where they rapidly undergo degeneration as new diverticula develop.

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# ISOPOD PARASITES OF FREE-LIVING COPEPODS OF MADRAS

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## INTRODUCTION

THOUGH the Bopyrids of the Indo-Pacific region have been studied by many workers and the Indian Bopyrids treated exhaustively by Chopra (1923), the Epicarids parasitic on free-living Copepods of the Indian coast have not received any attention. Nierstrasz and Brandis (1923) have given an excellent account of the Indo-Pacific Epicaridea collected by Siboga expedition, but the Microniscidæ parasitic on Copepods have been given only a brief reference. Tattersall's account (1905) of the Microniscidæ collected from Nordisches Plankton has however proved very helpful to the authors in preparing this brief note on three of the Indian forms, occurring on the Madras coast.

In the course of the studies of Copepods of Madras Plankton, 13 Isopods were found as ecto-parasites on free-living Copepods belonging to the genera *Acartia*, *Acrocalanus* and *Eucalanus*, between 11-9-1947 and 6-10-1947. The data collected as regards the number of parasites, their respective hosts, size and colour are given in the table below.

Type	No. of Specimen	Colour	Length	Breadth	Host	Remarks
A	2	Greenish-White with phosphorescent spots	mm. 1.64	mm. 0.9	Acartia	
B	1	Reddish	1.32 1.00 1.20	0.48 0.28 0.32	Eucalanus Acartia Acrocalanus	
C	7	Greenish-White	1.24 1.32 1.36 1.40 1.24 1.48	0.36 0.48 0.48 0.56 0.32 0.60		This is found parasitic on two hosts

The hosts were free-living Copepods belonging to the genera *Acartia*, *Acrocalanus* and *Eucalanus*. The parasites do not seem to affect the host

at all, as far as its swimming powers, body size or sexual maturity are concerned. Nor were there any pathological developments. The percentage of occurrence of the infected Copepods is very negligible. Though a total number of 6,000 specimens of *Acartia* was examined from 20 samples, the number of infected Copepods was only 7. In the same way, among the 10,000 *Eucalanus* and 8,000 *Acrocalanus* examined, only one of the former and two of the latter were found infected.

The 13 parasites collected fall into three definite types and appear to be restricted to three genera of hosts, *Acartia*, *Acrocalanus* and *Eucalanus*, one of the three types being found on two of these hosts (*Acartia* and *Acrocalanus*). As there were no means of determining whether these were larvæ or adults, male or female, the authors felt that they may be provisionally described as different species of the genus *Microniscus* with the specific names indicating the genera of the hosts, as *Tattersall* has done, until we know more about their life-histories.

All the three parasites were found attached to the cephalothorax of their hosts in the same oblique position, the posterior part of the parasite being on the lateral side of the host with the anterior side extending over the dorsal side. This position is probably due to the small size of the hosts—average length 2.97 mm., breadth 0.72 mm., in proportion to that of the parasites—average length 1.45 mm. and breadth 0.56 mm. It is also probable that the oblique position is the most suited for the parasite to be attached without hampering the movements of the limbs of the hosts. Prehension appears to be with the help of the antennæ, the thoracic legs, the flat form of the body and pointed uropods. The parasites are easily dislodged from the body of their hosts and when detached they crawl on their thoracic legs in the watch-glass. Frequently they fall on their dorsal side and remain inactive. Even when the parasites were crawling about, they appeared to be indifferent in securing a fresh host though several Copepods of their host genera as well as others were placed in their vicinity. After dislodging the parasite, the host was examined carefully for any injury inflicted by the mouth parts of the parasite. As there were no wounds in the few forms examined, it is possible that the parasites had just fastened themselves to their hosts. By the prehensile nature of the limbs and absence of food-capturing organs, the possibility of their being just epizoid can be ruled out. The parasites appear to have no power of swimming, the pleopods which appear to be respiratory never being brought into play. The entire abdomen not being attached to the host, the posterior free part was lifted up and lowered down. Such ventilatory movements were slow, lasting from 8 to 10 seconds and

repeated 5 to 7 times a minute. Observation of the tiny parasites was facilitated by staining them in neutral red. One of them moulted during observation, the ecdysis starting from the cephalic region and extending backwards. But it was found impossible to rear them for a period longer than three days.

#### TYPE A

##### *Microniscus acartii*

*Host:* *Acartia erythræa* Giesbrecht.

*Locality:* Madras Coast.

*Size of the Host:* 2-7 mm. long and 0.72 mm. broad (average).

The two specimens found, measured 1.64 mm. from the anterior tip of the cephalic plate to the posterior tip of the setæ of the uropod and 0.9 mm. across the body between the outer edges of the tergal plates. The body is twisted about the commencement of the abdominal region so that when the abdomen is viewed dorsally the part anterior to it is seen from a lateral aspect.

The segmentation of the body is very clear, especially on the ventral side. As in all isopods, there are seven free thoracic segments in front of which is a large and prominent shield of crescentic shape. This carapace covers the cephalothorax of six segments. The anterior edge of this plate projects freely forwards for a short length. This free, flexible margin is probably of use in getting a closer and firmer contact on the surface of the host's body than an abrupt sharp edge would secure. The dorsal side of the carapace is marked by a pair of conspicuous eyes consisting of 4 elements. The dorsal surface of the body is distinguished by two rows of 10 phosphorescent spots, one row on each side of the median line behind the carapace. Each spot is circular with irregular edges and is of a dirty yellow colour by daylight. The telson is a broad plate with a median emarginate tip (Fig. 1 T).

*Appendages.*—The first antenna extends ventrally beyond the carapace and is not hidden by it. The protopodite appears to be 3 jointed, cylindrical. The endopodite is short, slender and pointed—dactylose while the exopodite is two jointed, twice as long as the endopodite (Fig. 1 A). The second antenna is far longer, being nearly twice as long as the first. The three joints of the protopodite are cylindrical and the last two are marked by their distal edges being prolonged into a spine each (Fig. 1. B).

The mouth parts are attached medially just behind a rostral or labral plate which however does not form a complete sheath. The mandible, the 1st maxilla and the 2nd maxilla are attached closely together with their

distal tips directed inwards and forwards. The mandible appears to be simple, blunt-tipped, and probably with a sharp posterior edge. The tips of the two mandibles are directed medially. The 1st maxilla is three-jointed, the basal joints are stout while the third is long, slender and pointed. The 2nd maxilla is two-jointed and tapers into a pointed process (Fig. 1 D).

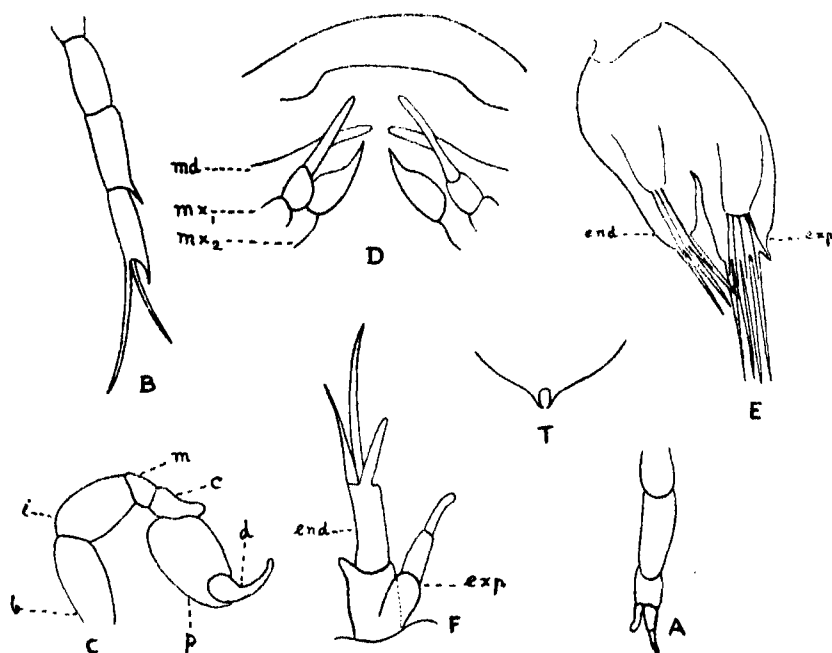


FIG. 1. *M. acartii* (Appendages)

As in all Isopods, there are seven pairs of six articulated thoracic legs. The legs are short and prehensile. The proximal part curves outward, while the distal part, ending in a long, slender claw-like dactylus, is curved inwards (Fig. 1 C). This facilitates a purchase on the host's body. There are five pairs of biramous foliaceous pleopods which are all alike and exhibit no trace of articulation. The exopodite of each bears a short, blunt spine at its outer distal edge and four setae. The endopodite bears three setae (Fig. 1 E). The uropod on either side is extended straight behind and is parallel and biramous. The protopodite is single-jointed bearing the endopodite on its inner side a little before its distal edge which is drawn out into a small spine on the outer side and bears the exopodite. The exopodite is long, cylindrical and bears three spines, the middle one being the longest. The endopodite is shorter and more slender. It is single-jointed and bears a spine (Fig. 1 F). The two uropods with their tapering spiny ramii are

pressed close together, to form the pointed, posterior end of the body and serve as a hold on the host.

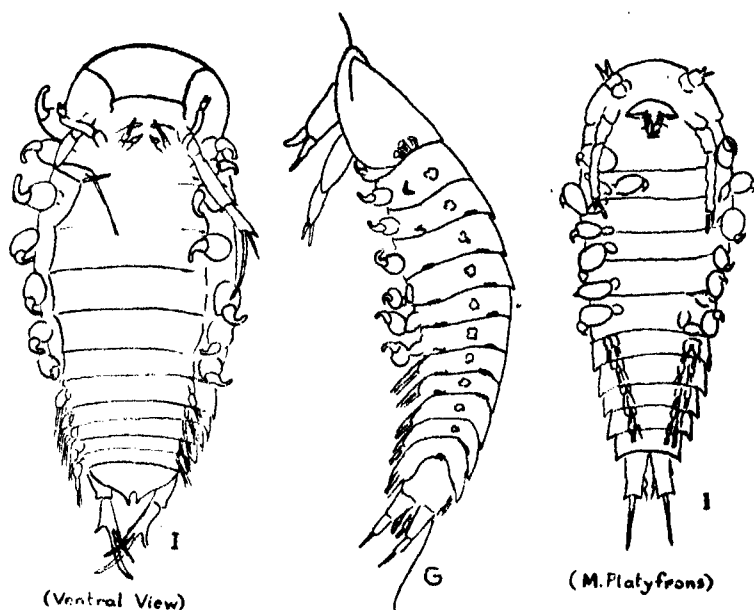


FIG. 2. Ventral and lateral views of *M. acartii* and ventral view of *M. platyfrons*

#### TYPE B

##### *Microniscus eucalani*

*Host:* *Eucalanus crassus* Griesbrecht.

*Locality:* Madras Coast.

*Size of Host:* 4.0 mm.

The parasite measured 1.32 mm. long and 0.48 mm. broad. The whole body is hirsute on the dorsal surface. There is no torsion of the body which is marked by a number of densely scattered red pigment spots giving the parasite a very light reddish colour. The edge of the cephalic plate is toothed and has no free flexible margin.

*Appendages.*—The 1st antenna is short, 3-jointed and uniramous. The basipodite has 3 spinous processes on its outer side. The 2nd joint is half as broad as the 1st and bears 3 setæ on its inner surface and a tuft of hairs on the outer side. The 3rd joint has 4 terminal bristles (Fig. 3 A). The 2nd (Fig. 3 B) antenna is nearly 5 times longer than the 1st. It is 8-jointed and flagellar. Each joint bears one or two spines on its distal edge while the terminal joint bears three long bristles.



The mouth parts are similar to those of *M. acartii*, excepting for the difference in the lengths of the joints of the 2nd maxilla. In the present form all the mouth parts are directed anteriorly (Fig. 3 D).

The thoracic legs appear better fitted for prehension. The propodus is large and swollen, with the sharp-pointed dactylus folding on it giving the leg a chelate termination (Fig. 3 C).

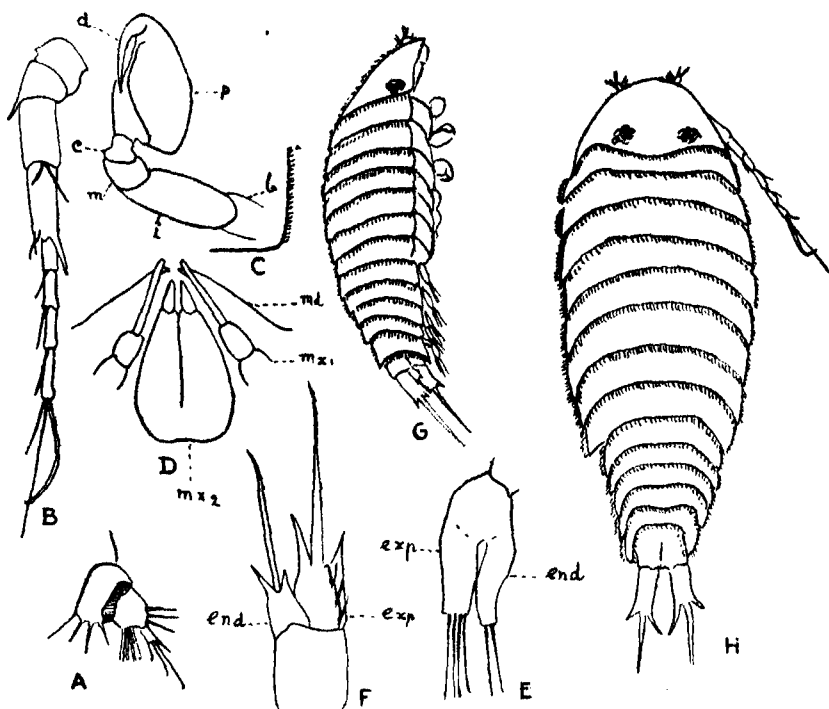


FIG. 3. *M. eucalanii* (Appendages and dorsal and lateral view)

There are five pairs of biramous pleopods. The endopodite bears two long setæ and the exopodite bears four long setæ (Fig. 3 E).

The uropods are biramous. The protopodite is single-jointed, stout, cylindrical. The exopodite has a sharp ridge bearing three slender spines and ends in a long curved spine.

The sides of the joint are produced into two stout, short spines, one on either side. The endopodite is smaller but single jointed. Its distal edge is drawn into two short stout spines, while it terminates in a long stout spine (Fig. 3 E).

Only one specimen of this type was found—though 20 samples each containing roughly 500 forms were examined.

## TYPE C

*Microniscus latyfrons*

*Hosts:* *Acartis erythræa* and *Acrocalanus longicornis* Giesbrecht.

*Locality:* Madras Coast.

*Size of Host:* *Acrocalanus*: 1.42 mm. long. *Acartia*: 3 mm. long.

Out of the eight specimens collected, 6 were parasitic on *Acartia* and two on *Acrocalanus*. These measured 1.36 mm. long and 0.48 mm. broad. The carapace of this form differs in having the anterior region depressed so that the front edge is flatter.

*Appendages.*—The 1st antenna (Fig. 4 A) is biramous and short. The protopodite appears to be two-jointed, the proximal joint being very large

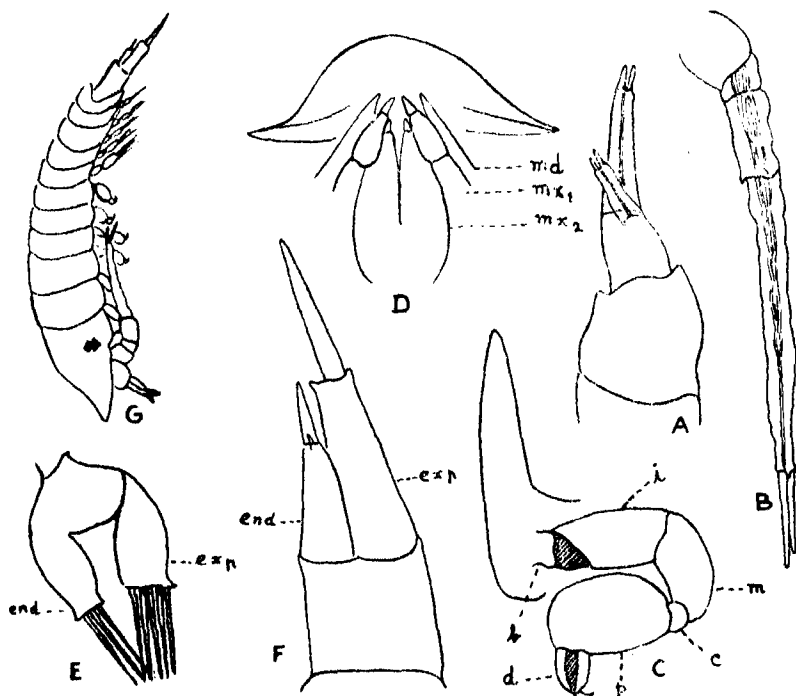


FIG. 4. *M. latyfrons* (Appendages and lateral view)

and stout. The second joint is about half its size, but longer and three times as stout as the endopodite. The exopodite is single jointed but twice as long as the endopodite. The endopodite is single-jointed, slender and ends in a pair of finger-like processes. The 2nd antenna (Fig. 4 B) is four-articled and three times as long as the 1st, uniformly thin and bears two small pointed processes at its tip.

The mouth parts (Fig. 4 D) are similar to those of the previous forms as regards their arrangement and structure. The difference in the lengths of the joints of the 1st maxilla and 2nd may however be mentioned.

There are seven pairs of six-jointed thoracic legs. The dactylus bears a small, curved pointed, process which helps in clinging. The propodus is large and rounded (Fig. 4 C).

There are five pairs of biramous pleopods. The exopodite has a small spinous process at its outer edge and bears five setæ. The endopodite has three setæ (Fig 4 E).

There is a pair of biramous uropod. The protopodite is two-jointed, cylindrical. The endopodite is single jointed and bears a stout spine half as long as the base. The endopodite is distinguished by a small slender accessory spine. The exopodite also consists of a basal piece and a spine and the basal portion is as long as the entire endopodite and is proportionately stouter. The spine is also more than twice the length of the spine of the endopodite (Fig. 4 F).

#### ACKNOWLEDGEMENT

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#### KEY TO LETTERING

A. 1st antenna.	M. Mandible.
B. 2nd antenna.	Max. 1. 1st Maxilla.
C. Thoracic leg.	Max. 2. Maxilla 2.
d. Dactylus.	E. Pleopod.
p. Propodus.	endp. Endopodite.
c. Carpus.	F. Uropod.
m. Merus.	exop. Exopodite.
i. Ischium.	endp. Endopodite.
b. Basis.	exop. Exopodite.
D. Mouth-parts.	T. Telson.

# THE EFFECT OF ASPHYXIA ON THE MECHANICAL RESPONSE OF THE FROG'S UNSTRIATED MUSCLE AND ITS RELATION TO TONUS

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THERE are very few observations on record on the metabolism of frog's unstriated muscle, comparable to similar ones on the frog's heart (Clark, *et al.*, 1938); for reference to work on mammalian unstriated muscle, see Prasad (1935 *a, b*, 1936). Saiki (1908) found that there was very little glycogen in the frog's stomach and bladder and that the lactic acid content in fresh specimens was about 0.06 p.c. Lovatt Evans (1925) found that the lactic acid content of frog's stomach in the resting state was 0.03 p.c. and after stimulation at 20° C. at intervals of 45 sec. for 2 hours, it was 0.04 p.c.; this does not show any significant increase. The oxygen consumption of the frog's stomach muscle was found by Rao and Singh (1940) to be 0.015 c.c./g. per minute ( $QO_2 \times 100 = 16.2$ ); this is about the same as that for frog's heart (0.0133 c.c.; Clark, *et al.*, 1938). Stimulation with alternating current once every 15 min. doubled the oxygen consumption.

In the present research the effect of asphyxia on the mechanical response of the frog's stomach muscle was investigated; the chemical estimations will be reported in a later paper.

## EXPERIMENTAL

In these experiments, unstriated muscle from the frog's stomach has been used (Singh, 1939). The cessation of oxygen supply to the muscle was produced by bubbling hydrogen. Either spontaneous activity was recorded, or the muscle was stimulated by alternating current, 8 volts/10 sec. per minute. It was also stimulated with potassium (0.02 M KCl), or by acetylcholine (1 in 10,000).

## RESULTS

*The stimulant effect of asphyxia.*—When asphyxiated or treated with sodium cyanide (1 in 10,000), frog's unstriated muscle differs from the frog's heart, in that the former shows a phase of increased excitability before final depression (Fig. 1). After the preliminary phase of increased excitability is over, the contractions may remain fairly constant for a long time ( $\frac{1}{2}$  to 3 hours). Introduction of oxygen now causes a depression in excitability

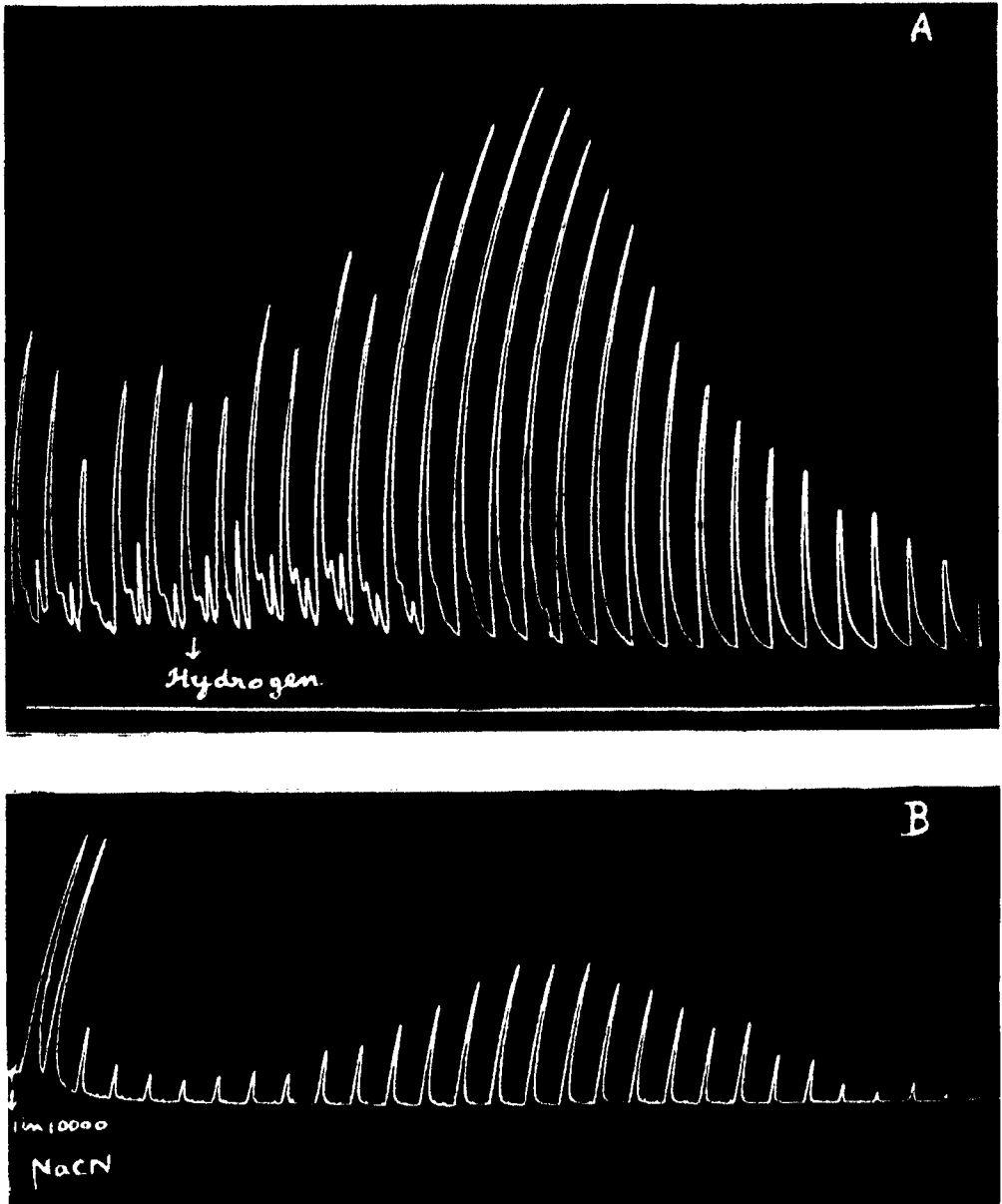


FIG. 1. Frog stomach muscle

- A. Asphyxial increase of excitability to alternating current
- B. Similar effect of NaCN, 1 in 10,000.

which may amount to complete paralysis (Fig. 2). This shows that in the muscle there are two mechanisms for obtaining energy, one aerobic and the

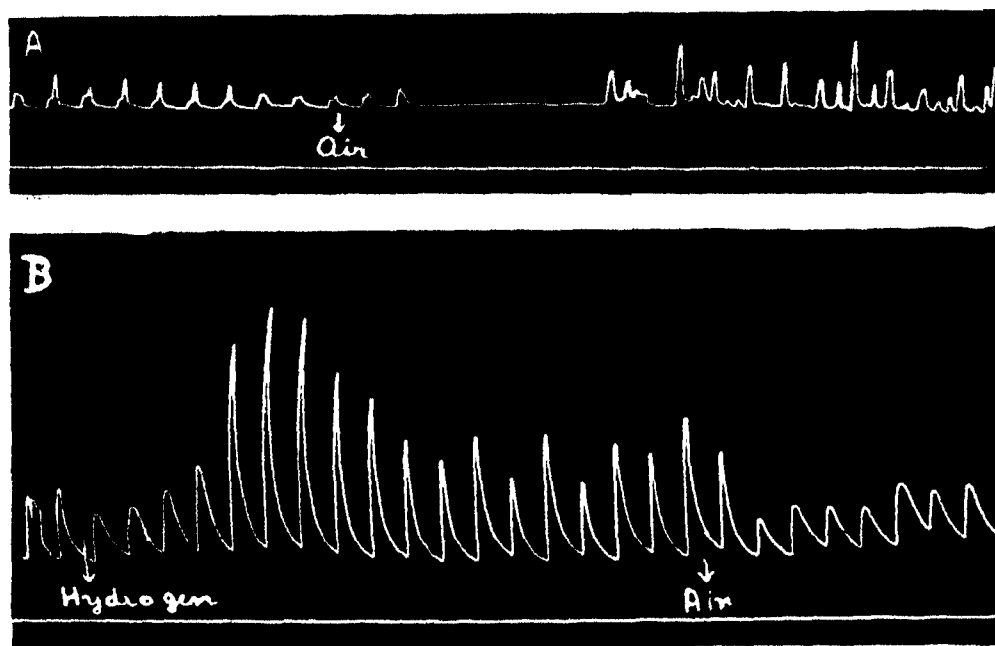


FIG. 2. Frog stomach muscle; stimulation by alternating current

- A. Asphyxiated muscle. Complete paralysis on introduction of air followed by recovery  
B. The effect of asphyxia and reintroduction of oxygen

other anærobic, which are mutually exclusive. These phenomena resemble those found in the chemoreceptors of the carotid body and in the nervous system. Thus the chemoreceptors of the carotid body are stimulated by oxygen lack and under certain conditions, their activity is depressed on the reintroduction of oxygen (Marshall and Rosenfeld, 1936). The stimulant effects of asphyxia in the central nervous system are well known (Lovatt Evans, 1945); reintroduction of oxygen may cause paralysis (Schmidt, 1941), Iodoacetic acid (1 in 20,000) does not abolish the asphyxial increase in excitability; this shows that it is not due to production of lactic acid. The optimum temperature for the increase in excitability is 30° C. (Fig. 3).

*Relief of asphyxial arrest.*—Asphyxial arrest is relieved by glucose in alkaline solutions and by substances that produce tonic contraction.

The stimulant action of glucose on the asphyxiated muscle is definite but it takes about 20–30 minutes for full development of the response, this presumably being the time required for diffusion of glucose. In the frog's heart, the action of glucose is more rapid, probably due to the fact that

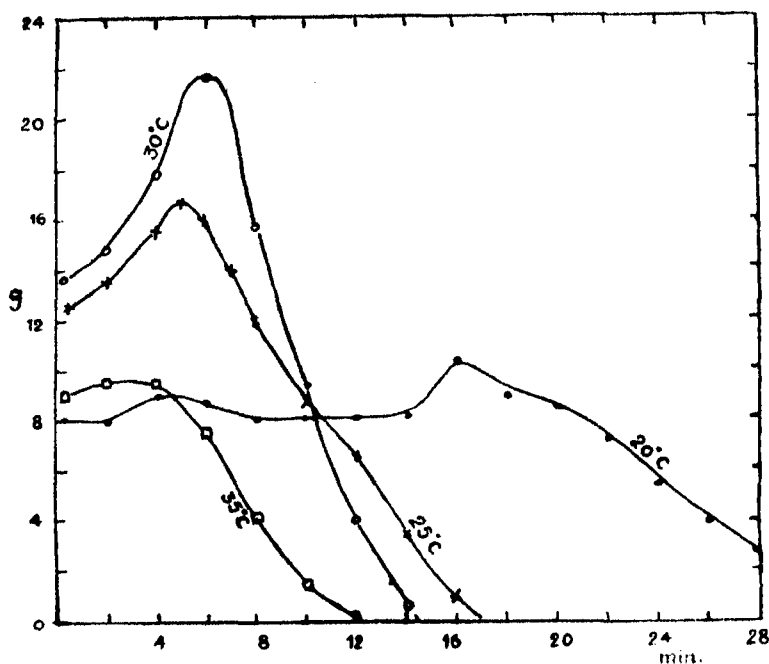


FIG. 3. Frog stomach muscle

Effect of temperature on the response of asphyxiated muscle to alternating current

diffusion is more rapid. As in the frog's heart, therefore, the stimulant action of any substance can be used to show whether it could be available as anærobic source of energy. The stimulant action must be permanent, as temporary changes lasting for 20-30 minutes might take place on introduction of any substance. The results are shown in Table I. The isolated muscle exhausted of carbohydrate by continued contraction in the absence of oxygen is revived by the addition to the perfusion fluid of glucose, but not of mannose, fructose, galactose, arabinose, xylose, maltose, lactose, sucrose, glycogen, starch, sodium lactate, glycine, asparagine, sodium acetate, sodium propionate, sodium butyrate, sodium oleate. Frog's unstriated muscle, thus differs from the frog's heart in that it cannot utilise mannose.

Other substances that relieve asphyxial arrest are those which produce tonic contraction, such as cations, Li,  $\text{NH}_4$ , K, Ba; anions,  $\text{NO}_3$ , I, SCN; drugs, eserine, acetylcholine. The power of these substances to relieve asphyxial arrest is proportional to their power in producing tonic contrac-

TABLE I

*Beneficial action of carbohydrates, etc., on (a) asphyxiated and exhausted frog's stomach muscle and (b) the asphyxiated and exhausted frog's heart*

Subject	(a) Asphyxiated and exhausted frog's stomach muscle	(b) Asphyxiated and exhausted frog's heart (Gaddie and Stewart, 1934)
Polysaccharides :		
Starch	..	..
Glycogen	..	—
Disaccharides :		
Maltose	..	—
Lactose	..	—
Sucrose	..	—
Monosaccharides :		
Glucose	..	+
Mannose	..	+
Fructose	..	—
Galactose	..	—
Pentoses :		
Arabinose	..	—
Xylose	..	—
Amino acids :		
Glycine	..	—
Lipoids :		
Sodium oleate	..	—

tion; potassium is the most powerful (Fig. 4). In contrast to the action of glucose, these substances are more effective in acid than in alkaline solutions, even at pH 6, and in the presence of iodoacetic acid. These experiments show that twitch and tonic contractions, besides differing from each other in several respects, as described in previous papers, also differ as regards their metabolism.

*Effect of asphyxia on tonus.*—Asphyxia has two effects on tone and tonic contractions mentioned above. At first tone decreases and then increases (Fig. 5). This asphyxial contraction can be maintained for several hours and is easily reversible, though after 12 hours, it becomes irreversible, and the condition resembles *rigor mortis* of striated muscle. These findings are in agreement with the oxygen consumption measurements (Rao and Singh, 1940). It was found that two kinds of tonic contractions were produced; during one the oxygen consumption increased, and during the other, it decreased.

It has been shown that substances that produce tonic contraction, retard the relaxation of unstriated muscle (Singh, 1938); in confirmity with this,



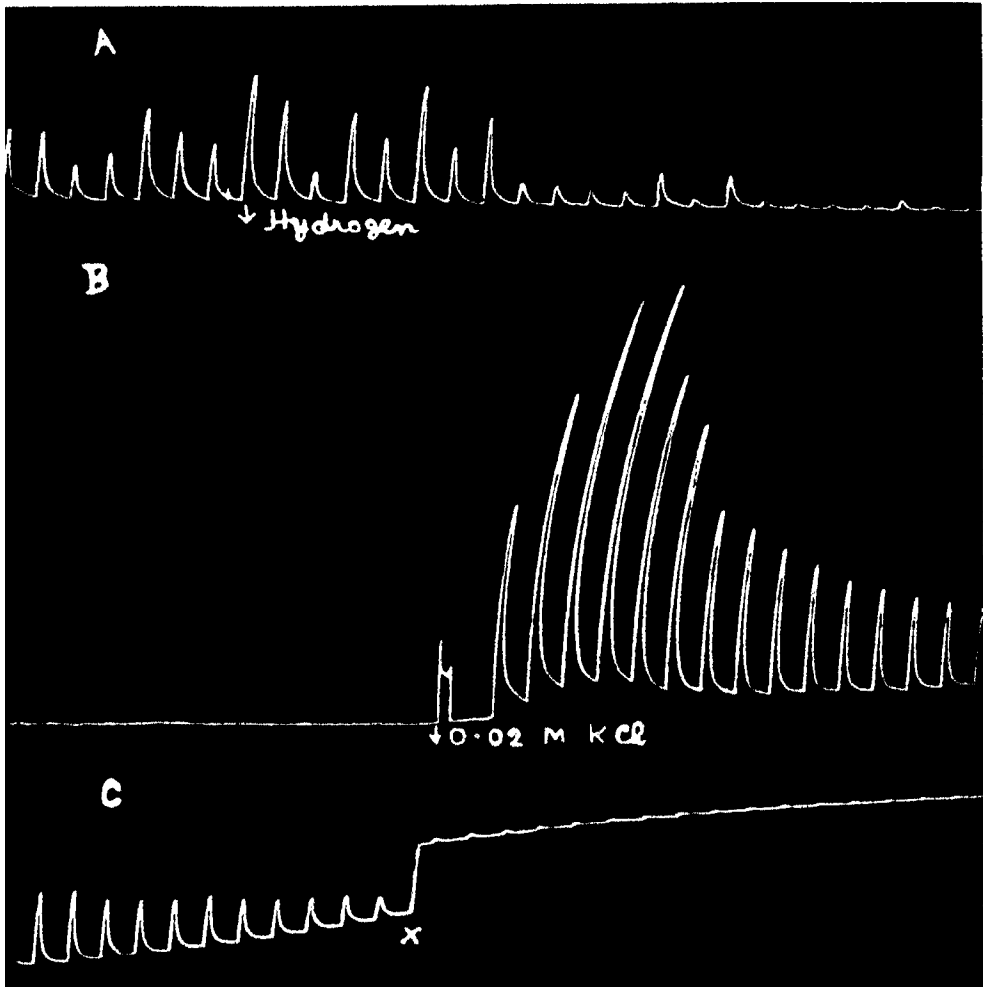


FIG. 4. Frog stomach muscle, pH 6

- A. Asphyxial arrest by hydrogen
- B. Relief of asphyxial arrest by potassium
- C. Above tracing continued. Drum stopped for 15 minutes at x. Note asphyxial contraction

it has been found that relaxation may be retarded as a result of asphyxia. Cyanide retards relaxation (Rao and Singh, 1940).

*Action of iodoacetic acid and acid solutions.*—The fact that the asphyxial exaltation of excitability is not suppressed by iodoacetic acid, causes frog's unstriated muscle to behave quite differently from frog's heart and mammalian unstriated muscle when deprived of oxygen, its movements being not rapidly

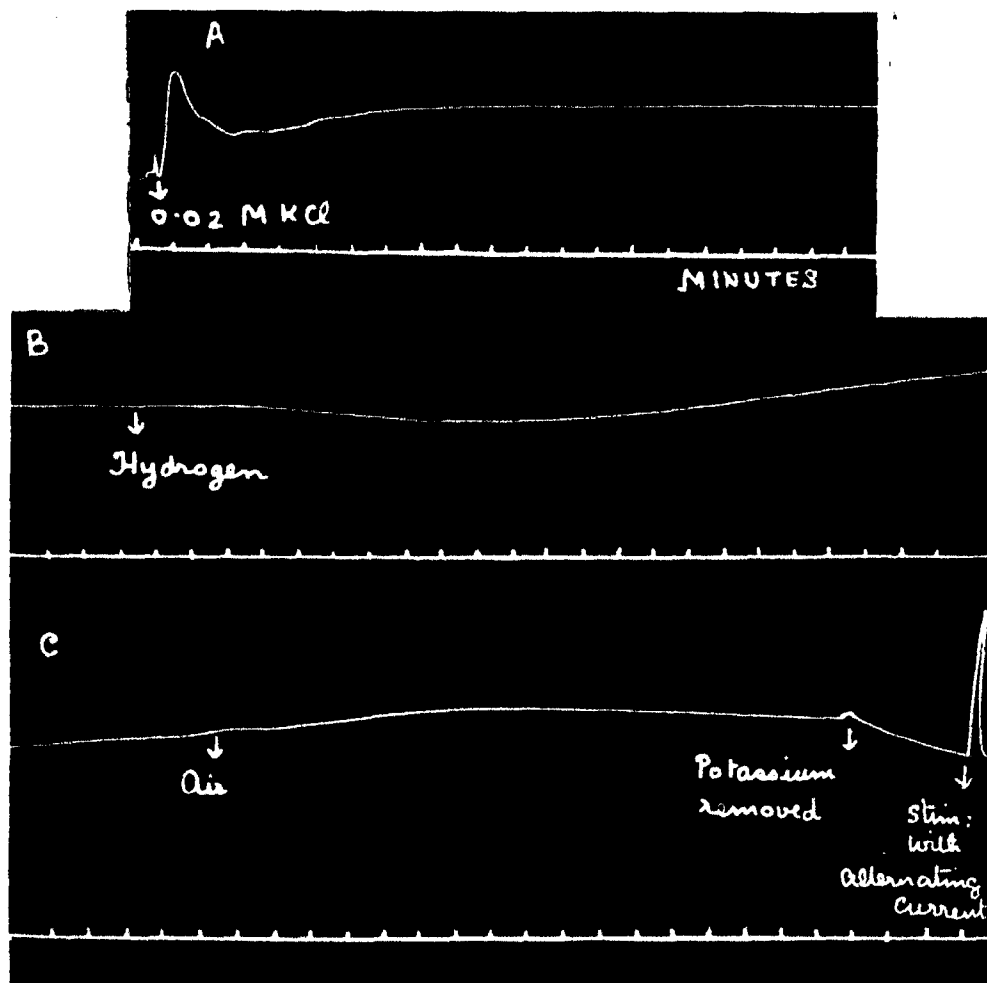


FIG. 5. Frog stomach muscle

- A. Stimulation by 0.02 M KCl
- B. When the response is steady hydrogen is introduced
- C. Introduction of air. After a few minutes tone begins to decrease. On removal of potassium, tone decreases and the muscle responds normally to alternating current

arrested. In a muscle poisoned with iodoacetic acid, asphyxia causes rapid arrest if the pH is raised to 6, as at this reaction, the asphyxial increase in excitability does not occur. These experiments show that though both iodoacetic acid and pH 6 suppress production of lactic acid, their actions are not identical.

If a muscle, poisoned with iodoacetic acid, is exhausted by stimulation in the absence of oxygen and then revived by oxygen, the response is never as big as before. As in the case of frog's heart, the response is improved by certain substances, which are sodium lactate and sodium butyrate. Glucose, glycine, sodium acetate and sodium propionate do not improve the response.

*In the presence of oxygen*, mammalian unstriate muscle becomes hyper-excitable in acid solution (Singh, 1940). Frog's unstriated muscle also becomes more sensitive in acid solutions. This suggests that the muscle has developed some other aerobic mechanism to deal with acid solutions, as the production of lactic acid suppressed by such solutions.

The effect of substances mentioned in connexion with the action of glucose, was also studied in an exhausted muscle at pH 6 in the presence of oxygen. It might be of significance in metabolism of the muscle in acid solutions. Glucose, mannose, fructose, galactose, sucrose, lactose, maltose, glycogen and starch had no effect, nor did glycine have any beneficial action. Sodium acetate, propionate improved the response, but not sodium butyrate or oleate. Sodium lactate improved the response.

### DISCUSSION

Frog's unstriated muscle differs from the frog's heart in many respects. It has several aerobic and anaerobic metabolic mechanisms. Thus it has a different aerobic mechanism to deal with acid solutions. It has an anaerobic mechanism, other than the glycogen-lactic acid system, and it has a separate mechanism for tonic contractions.

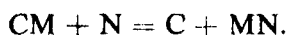
The fact that potassium releases some store of energy in a muscle poisoned with iodoacetic acid, may be of importance in the process of stimulation by electric current; the latter would cause movement of potassium ions inside the fibres and increase its concentration at some membrane. This would release the energy for contraction.

*A new theory of tonus.*—In asphyxial contraction is a mechanism whereby the muscle can maintain tension without expenditure of energy. It is therefore, reasonable to assume that tonus of muscle which requires no extra energy for maintenance would be identical with or related to the asphyxial contraction. The part of tonic contraction which does not require energy for maintenance is the slow relaxation; as slow relaxation occurs as a result of asphyxia or cyanide, the asphyxial contraction appears to be related to tonus that does not cause increase in oxygen usage. This is further supported by the fact that the oxygen consumption may diminish during

normal tonic contraction (Lovatt Evans; Rao and Singh) and increase during inhibition (Rao and Singh).

The asphyxial contraction suggests that the normal state of the myosin molecule is that of shortening. This would account for the shortening of muscle without increased oxygen consumption. How can, then, the other kind of shortening, which is accompanied by increase in oxygen consumption be accounted for? Our previous scheme (Singh and Singh, 1946) has to be amplified.

If MT, which is a compound of myosin (M) and another chemical substance T, represents the shortened molecule, then normally the effect of T is neutralised by some other substance C. MTC, then, may represent myosin in its relaxed state. Postulation of T however is not necessary; the relaxed molecule may be represented by MC. Contraction, then, can be caused by two processes: (i) If the influence of C upon M is removed; (ii) or if another substance combines with MC, say Q, which forms MCQ; in the first instance MC may be dissociated or split by some other substance N.



The maintenance of tension in this case will not require expenditure of energy. In the second instance, the compound MCQ or Q itself may be unstable, and so require continuous expenditure of energy. The tonic and twitch contractions will then be respectively represented by M and MCQ.

#### SUMMARY AND CONCLUSIONS

1. Asphyxia at first increases the response of frog's unstriated muscle; this is followed by diminution and then paralysis. These effects are also produced by cyanide. This increase in excitability is not abolished by iodoacetic acid.

2. Asphyxial arrest is relieved by glucose, potassium and the other substances that produce tonic contraction.

3. In the presence of oxygen the muscle becomes hyperirritable in acid solutions and so possesses different aerobic metabolic mechanisms in alkaline and acid solutions respectively.

4. In acid solutions, pH 6, sodium lactate, acetate and propionate improve the response to alternating current.

5. Asphyxia does not produce rapid arrest of movement in muscle poisoned with iodoacetic acid.

6. In a muscle poisoned with iodoacetic acid, then exhausted by frequent stimulation in asphyxia, and then revived by oxygen, sodium lactate

and sodium butyrate improve the response. Glucose, glycine, sodium acetate and propionate have no effect.

7. In asphyxia, tone at first decreases and then increases; this asphyxial contraction is identical with tone that does not use oxygen.

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# STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

## Part II. Reproduction in the Male Vespertilionid Bat *Scotophilus wroughtoni* (Thomas)

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### INTRODUCTION

A STUDY of the reproductive phenomena in bats offers a number of interesting features. In this paper an attempt is made to record the cyclical changes in the male reproductive organs in the breeding and the non-breeding seasons. As mentioned in the first part of this series (1947)<sup>3</sup> the study is based entirely on the examination of the wild specimens for the obvious reason that any experimental study is impossible in the caged bats. The specimens were all collected round about Bangalore, which is a tropical zone of more or less unvarying climatic conditions throughout the year. A general account of the breeding seasons of this species of bats has formed the subject-matter of the first part of this paper. It was based upon a gross study of the females only. The results of the study of the male reproductive organs are incorporated in this paper; and these confirm the conclusions already arrived at regarding the breeding seasons.

### HISTORICAL

Though a large volume of literature is available on the general breeding habits of the insectivorous bats, and also on the female sexual cycle of many species, it is a matter of considerable surprise that very little work has been done on the sex-cycle of the male bats. The little information we have on the subject of the reproductive processes of the male bats is the result of a study of the female genitalia for the presence of spermatozoa in the vaginal tract or the oviduct and the casual observations on the exaggerated secondary sexual characters in the male during the breeding season.

A study of the literature on bat reproduction reveals that in a majority of the species inhabiting the temperate and cold climates, the males experience a height of sexual activity during late autumn when they copulate, followed by hibernation during winter. The spermatozoa also undergo hibernation in the genital tract of the female and fertilize the ovum in the next spring. A large volume of circumstantial evidence has also accumulated to substantiate this view. Courrier (1927)<sup>2</sup> has shown in *Pipistrellus pipistrellus* that the examination of the male genitalia revealed the fact that after copulation

in late autumn the testes degenerated and throughout winter contained no spermatozoa, but only spermatogonia and sertoli cells. Nakano (1928)<sup>8</sup> working on *Vespertilio abramus* has come to similar conclusion that after copulation in October the testes degenerate. A very positive proof of autumn copulation was given by Harrison Matthews (1937)<sup>5</sup> working on the British Horse-shoe Bats. Because of the occurrence of a hard vaginal plug in the female throughout the winter after copulation he concludes "The ovum must have therefore been fertilized by one of the spermatozoa stored in the upper part of the genital tract and the spermatozoa must have been deposited in the previous autumn."

On the other hand, there are a few species of insectivorous bats in which copulation occurs in spring followed immediately by ovulation and fertilization resulting in pregnancy.

Baker and Bird (1936)<sup>1</sup> recorded in *Miniopterus australis* which is a tropical species that there is an annual sexual rhythm in the males which show sexual activity in July, August, September, and October (*i.e.*, the southern spring), and the testes would be nonfunctional during the rest of the year. They recorded as follows: "Looking at the material as a whole one sees clearly that copulation takes place about the end of August, and that the development of the embryo starts at once. Copulation occurs at a time of the year when the days begin to get longer and the temperature is rising." Recently, Harrison Matthews (1942)<sup>3</sup> recorded his observations on some of the South African bats in which copulation occurs in spring and is immediately followed by fertilization and pregnancy.

There is still a third kind of observation. Rolland, E. Miller (1939)<sup>7</sup> working on *Myotis lucifugus lucifugus* and *M. grisescens* observed that "Copulation is known to occur in the fall. The conditions of the accessory glands and the presence of sperms make recurrent copulations possible until spring." On examining the accessory reproductive glands he recognised that the glands were functional and the epididymis full of sperms throughout winter and spring. He was convinced that there was a period of spring copulation too. He is, however, not certain whether the ovum is fertilized by the sperm of spring copulation or fall copulation. Mary, J. Guthrie (1933)<sup>4</sup> recorded that spring copulation is a normal occurrence in some American cave bats. She believed that the spermatozoa stored in autumn copulation are destroyed by the numerous phagocytes of the genital tract and copulation occurs again in early spring.

It is evident from the foregoing summary that most of the work relates to bats of the temperate regions in which two very different types of observations are recorded. There is practically no work on tropical bats except

that of Baker and Bird (1936)<sup>1</sup> and recently of Harrison Matthews (1942).<sup>6</sup> Both these authors observe that copulation takes place in spring and is immediately followed by fertilization and pregnancy.

#### MATERIAL AND METHODS

Bats were collected from the hollows of the trees in forests round about Bangalore. They were killed by chloroform and immediately dissected. Unfortunately their body weights were not taken. The genital organs, kidneys and suprarenals were fixed. Bouin's picro-formal-acetic was the usual fixative used. But other fixatives like Sanfelice's formula and corrosive acetic were also used. The weight of the testis and the epididymis was taken together after preservation in 70% alcohol. They were sectioned and stained in hæmatoxylin. The measurements of the diameters of the testis tubules and the tubules of the epididymis were taken by the use of a micrometer scale.

Table I gives the data of collections of the males and females. I began collections in May 1945 and it is still in progress.

TABLE I

*Monthly record of collections: Scotophilus wroughtoni* (Thomas)

Month	Date	Males	Females
May	17-5-1945	2	28
"	7-5-1946	2	2
June	7-6-1946	2	2
July	..	No collections	
August	..	do	
September	18-9-1946	1	1
October	20-10-1945	1	1
November	8-11-1945	2	2
December	7-12-1946	2	17
January	8-1-1947	3	5
February	10-2-1946	5	3
March	22-3-1946	2	4
April	1-4-1946	1	6
"	22-4-1946	4	26
"	28-4-1947	1	9
"	29-4-1946	1	20
Total	..	29	126

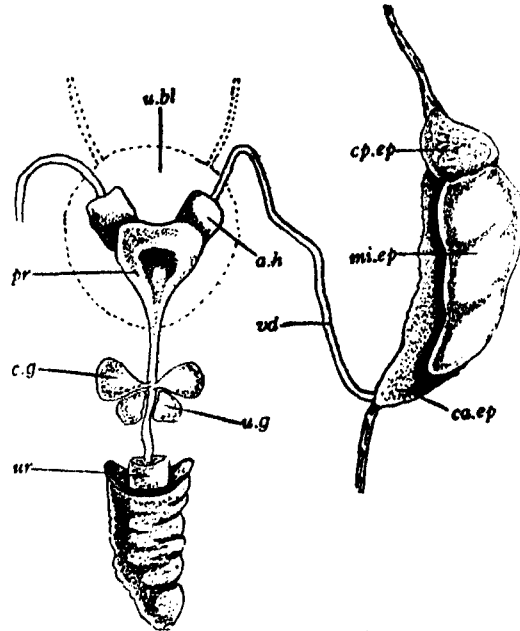
The numbers do not give any idea of the sex-ratio or the population of these animals, primarily because of the small number of specimens collected and secondly because of the probability that the males and the females must have lived separately after the active breeding period. It is worth noticing, however, that during January and February the proportion of males to females is markedly higher than during the other months. This may probably roughly indicate that during this period the males and the females live together.



## OBSERVATIONS

(A) *The Male Reproductive Organs* (Text-Fig. 1)

The male reproductive system consists of a pair of testes whose position and size varies during the different seasons of the year. The testes are abdominal in position during the non-breeding periods and becomes inguinal



TEXT-FIG. 1. Reproductive system of *Scotophilus wroughtoni* (Thomas)  $\times$  circa 6. *a.h.* ampulla of Henle; *ca.ep.*, cauda epididymis; *c.g.*, Cowper's gland; *cp.ep.*, caput epididymis; *mi.ep.*, mid-epididymis; *pr.*, prostate; *u.bl.*, urinary bladder; *u.g.*, urethral gland; *ur.*, urethra; *vd.*, vas deferens.

and sometimes even post-anal at the height of sexual activity, consequent not only upon their migration but also upon their enlargement. Each testis is slightly tapering towards the caudal end.

The epididymis is not very clearly demarked into a caput (*cp.ep.*), a mid (*mi.ep.*), and a cauda (*ca.ep.*) epididymis. The names are given to the portions of the epididymis in the figure by reason of the position. But on the onset of the breeding season the cauda epididymis (*ca.ep.*) is much elongated. The vas deferens (*vd.*) arises from the cauda epididymis (*ca.ep.*) and passes upwards on the median side of the testes between it and the bladder (*u.bl.*). Each vas deferens at its distal end is swollen up into a bean-shaped structure—the ampulla of Henle (*a.h.*). A true seminal vesicle distinct from the ampulla of Henle is absent. Thus the structures bear resemblance to some of the South African bats described by Harrison Matthews (1942).<sup>6</sup> The ampullæ of Henle are embedded in the wall of the

prostate glands (*pr.*) for nearly two-thirds of their length as in *Miniopterus minor* and *M. dasythrix* (Harrison Matthews, 1942).<sup>6</sup> The prostate is a fairly large structure lying below the bladder. The urethra (*ur.*) a little down below the prostate is joined by a pair of small pear-shaped glands—the Cowper's glands (*c.g.*). These lie just above the rectum. Posterior to the Cowper's glands are a pair of urethral glands (*u.g.*). The penis is directed caudally.

### (B) Seasonal Variations in Testis

#### (i) Macroscopic Examination

Each testis was weighed along with its epididymis. Table II gives these weights for the several months of the year. It is a well-known fact that in

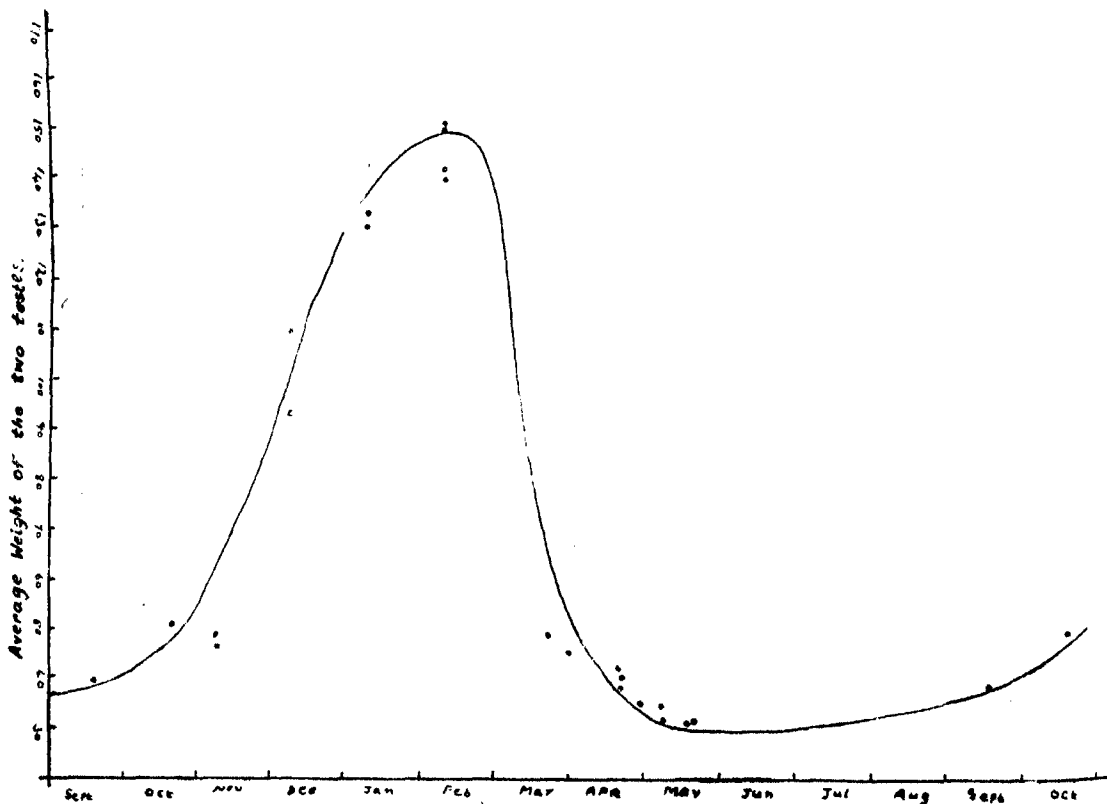
TABLE II

*Scotophilus wroughtoni* (Thomas)—Macroscopic and Microscopic Measurements of the Testes and the Epididymides

Month	Date	Index No.	Weight of the testes and epididymides in milligrammes				Diameters of the seminiferous tubules (in microns)			Diameters of the epididymal tubules (in microns)		
			Right	Left	Total	Average	Smallest	Largest	Average of 10	Smallest	Largest	Average of 10
January ..	8-1-47	123	145	142	287	143.5	156	170	160	93	114	101
do	do	128	130	136	266	133.0	..	..	..	..	..	..
do	do	129	132	128	260	130.0	140	170	151	107	114	108
February..	10-2-46	32(i)	143	140	283	141.5	..	..	..	..	..	..
do	do	32(v)	..	..	..	..	..	..	..	..	..	..
do	do	32(vi)	150	152	302	151.0	171	189	178	107	128	120
do	do	32(vii)	135	144	279	139.5	167	186	174	110	128	118
do	do	32(viii)	148	153	301	150.5	..	..	..	..	..	..
March ..	22-3-46	33(c)	48	51	99	49.5	..	..	..	..	..	..
do	do	33(d)	52	53	105	52.5	78	111	101	70	104	78
April ..	1-4-46	45	46	46	92	46.0	..	..	..	..	..	..
do	22-4-46	57	42	40	82	41.0	68	93	87	54	68	62
do	do	63	40	42	82	41.0	..	..	..	..	..	..
do	do	64	38	47	85	42.5	..	..	..	..	..	..
do	do	66	40	36	76	38.0	..	..	..	..	..	..
do	28-4-47	138	36	38	74	37.0	..	..	..	..	..	..
do	20-4-46	88	38	33	71	35.5	..	..	..	..	..	..
May ..	7-5-46	100	32	33	65	32.5	57	86	69	29	50	38
do	do	101	36	..	..	36.0	..	..	..	..	..	..
do	17-5-45	9	34	30	64	32.0	..	..	..	..	..	..
do	do	27	33	29	62	31.0	58	78	64	29	48	38
June ..	7-6-46	108(a)	..	..	..	..	..	..	..	..	..	..
do	do	111(f)	..	..	..	..	61	86	68	24	41	36
July	No specimens were available and all the expeditions for collections were fruitless											
August												
September	18-9-46	113(a)	38	41	79	39.5	71	93	80	43	80	56
October	20-10-45	30(a)	49	51	100	50.0	73	106	96	50	73	61
November	8-11-45	31(c)	47	50	97	48.5	..	..	..	..	..	..
do	do	31(d)	46	48	92	46.0	114	143	120	64	93	81
December	7-12-46	106(a)	90	96	186	93.0	134	161	150	93	114	100
do	do	118(b)	107	112	219	109.5	121	157	144	71	100	92

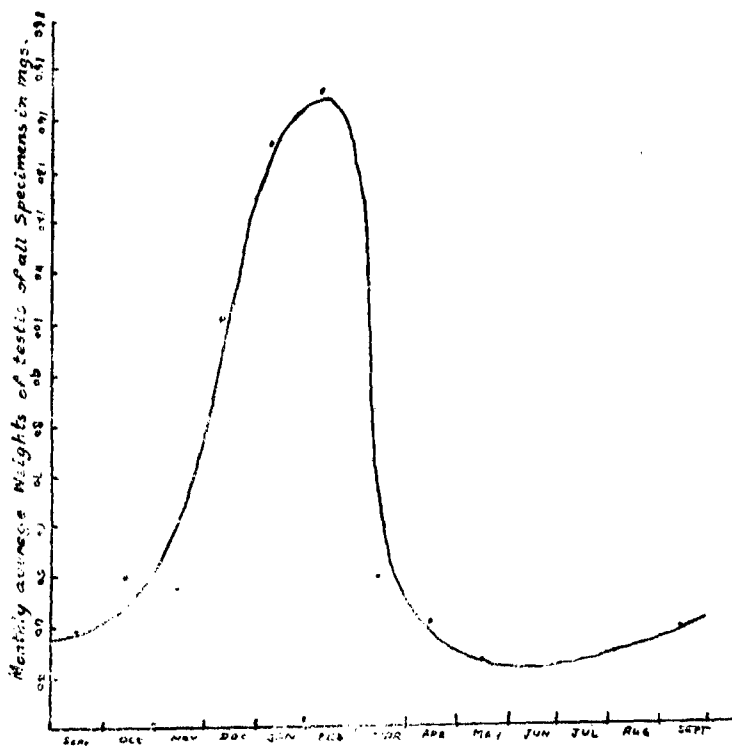
the case of the insectivorous bats the growth of the young is very rapid and furthermore, unlike the bats of the temperate and cold climates all the males were fecund during the breeding seasons.

Text-Fig. 2 is a graph of the average weights of the testis and epididymides of the two sides plotted against the months. Text Fig. 3 gives the graph of the weights of the testes average of all specimens of the month plotted against the months.



TEXT-FIG. 2. Graph to indicate the increase in the weight of the testis during the breeding season. The average weight in milligrammes of the two testes of each specimen is plotted against the different months of the year. There is an enormous increase in the weight of the testes during the months of January and February.

The graphs clearly indicate that the weight of the testis increases rapidly during the months of December, January and February, and suddenly decreases during the month of March, after copulation, and the following months till September. The weight begins to increase from October onwards which marks the onset of sexual activity. The February testis is nearly four times as heavy as the testis of September.

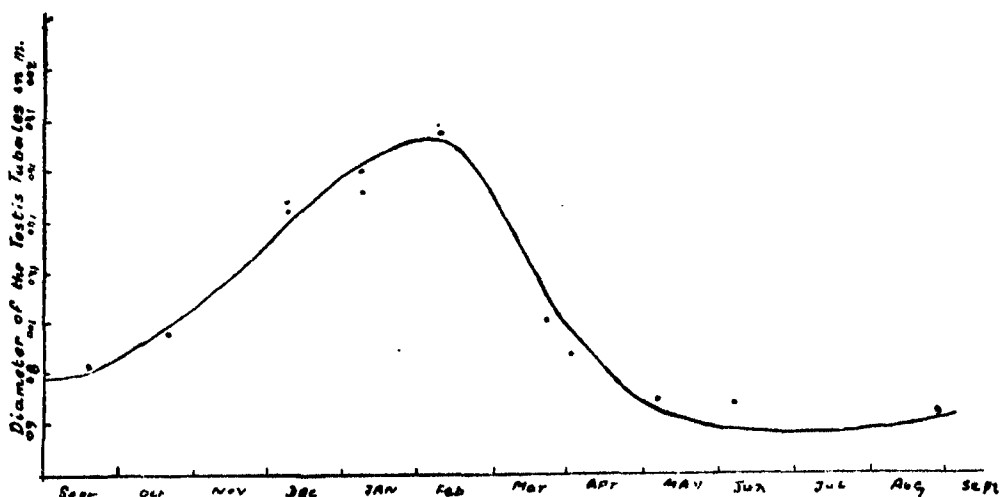


TEXT-FIG. 3. Graph of the average weight of the testes of all specimens of each collection plotted against the months.

## (ii) *Microscopic Examination*

(a) *Diameter of the seminiferous tubules.*—The size of the seminiferous tubules varies during the several months of the year according to the activity of the testis. Table II gives the diameters of the seminiferous tubules during the different months of the year. For the sake of convenience the measurements of the seminiferous tubules of only one specimen from the collection of each month is taken. There is no marked difference among the testis tubules of the several specimens of any one collection. Diameters of ten tubules are taken, five situated near the periphery and five near the centre and a mean value is arrived at. Text-Fig. 4 is a graph representing the variation in the diameter of the seminiferous tubules from the specimens collected during the different months of the year.

The mean diameter which was only  $80\mu$  in September suddenly increases on the onset of the activity of the germinal epithelium and increases to  $150\mu$  during December and January, and reaches a maximum diameter of  $180\mu$



TEXT-FIG. 4. Graph to indicate the increase in the diameter of the tubules of the testes during the breeding season. There is a considerable increase in the diameter of the tubules during the months of January and February. The diameters are taken in microns.

in February (10-2-46). As already remarked the testes of the several specimens of any one collection show similar seminiferous tubules. It is an important fact to note as it gives us a possible age of the specimens at which the male bats become sexually mature.

During March there is a sudden decrease in the diameter of the seminiferous tubules, and the tubules of May specimens show a small diameter of  $60\mu$  to  $70\mu$ , and very much the same as the September testis.

The diameter of the testis tubules seems steadily to increase with the weight of the testis. Text-Fig. 6 is a graph to show the relationship of the diameter of the testis tubules and the weight of the testis.

(b) *Histological changes in the testis*.—The microscopic examination of the sections of the testis indicates that in the specimens collected between the months of April to November, the tubules are inactive showing the germinal epithelium cells with resting nuclei. The tubules have no lumina, and the intercellular spaces are large. But in specimens collected in December the tubules have sprung up to activity showing plenty of mitotic figures. The spermatogenetic activity reaches its maximum vigour during January and February.

In May the testis tubules are small and have no lumina (Plate IV, Fig. 1). The tubules are composed of resting spermatogonia and sertoli cells. The nuclei of the spermatogonia are large, spherical and lightly stained and the individual cells are clearly marked out. The sertoli cells

are not so distinctly marked out. Their nuclei are small, are more densely stained, and they appear to be floating in the sertoli syncytium.

The sections do not show the presence of many capillaries.

The condition of the testis remains more or less the same till September. The testis tubules are still without lumina, and the sertoli cells are small and darkly stained. There are two or three layers of spermatogonia at the periphery with large spherical nuclei. Some spermatogonia are undergoing division. The intertubular spaces are still large. The vascularisation of the testis is still low.

The testes of November collection show lumina in a few of the peripheral tubules, but the tubules in the centre are still without lumina. The intertubular area is reduced. The tubules contain mainly the darkly staining spermatocytes and the sertoli cells, which are few in number.

In December the testis tubules have greatly enlarged and most of the tubules have clear lumina. The germinal epithelium is active showing all stages of spermatogenesis (Plate V, Fig. 3). The peripheral part of the tubule contains a few spermatogonia with large faint nuclei abutted against the wall of the tubule and very few in number. But the majority of the cells are spermatocytes in various stages of spermatogenesis. Towards the central part of the tubule there are a large number of spermatid nuclei, which are characterised not only by their small size but also by their darkly staining nuclei. A few of the spermatids have undergone spermateleosis and consequently the lumen presents even ripe spermatozoa. The sertoli cells are reduced in number.

In January and February the testis is in full swing of activity with large numbers of meiotic nuclei (Plate V, Fig. 4 and Plate VI, Fig. 6). The lumen of the tubule is enlarged and is packed with spermatozoa. All the spermatids are undergoing the final change and large swarms of sperms are found radiating towards the centre.

As the tubules have greatly hypertrophied the intertubular space is reduced to a minimum.

The vascularisation of the testis is markedly high and a number of capillaries are seen in the sections, both in the mass of the testis and also in the walls.

### *(C) Seasonal Variations in the Epididymis*

The epididymis also shows a seasonal variation corresponding to the variation in the testis. Table II gives the data regarding the variation in the diameter of the epididymal tubules during the different months of the year.

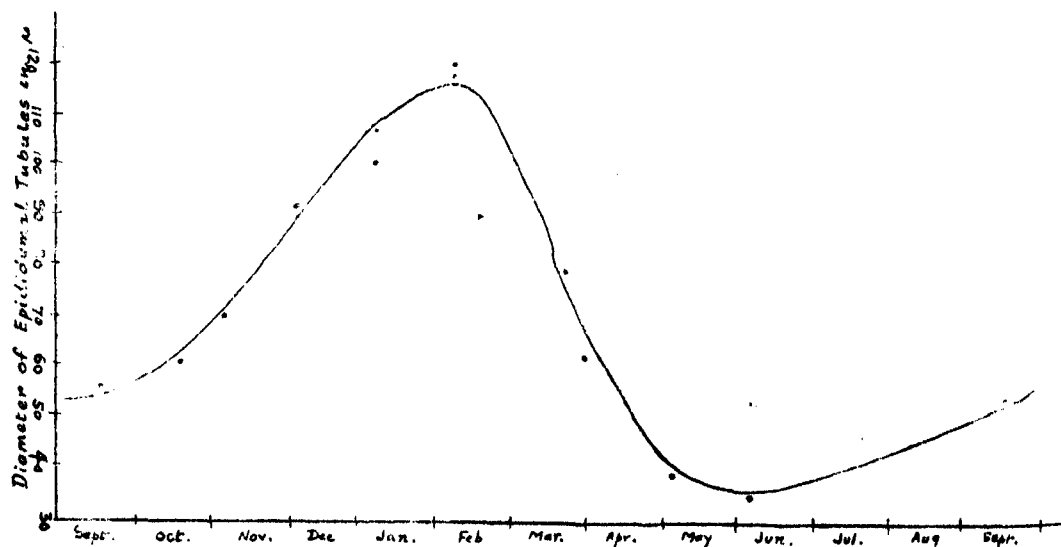
The tubules which were very narrow during the months between May and September, widen out from November onwards. The epithelium which appears to be two or three layered (due probably to shrinkage) flattens out into a single layered cubical epithelium.

From May to September there does not seem to be any change in the diameter of the tubules. It is narrow and varies from  $29\mu$  to  $43\mu$  in May to  $43\mu$  to  $60\mu$  in September. The intertubular area is large and is traversed by connective tissue and circular muscles (Plate IV, Fig. 2).

During November the diameter of the tubules is already large ( $57$  to  $64\mu$ ). And in December the diameter increases to  $100\mu$  to  $114\mu$  and the intertubular spaces are much reduced. Furthermore the lumina contain spermatozoa.

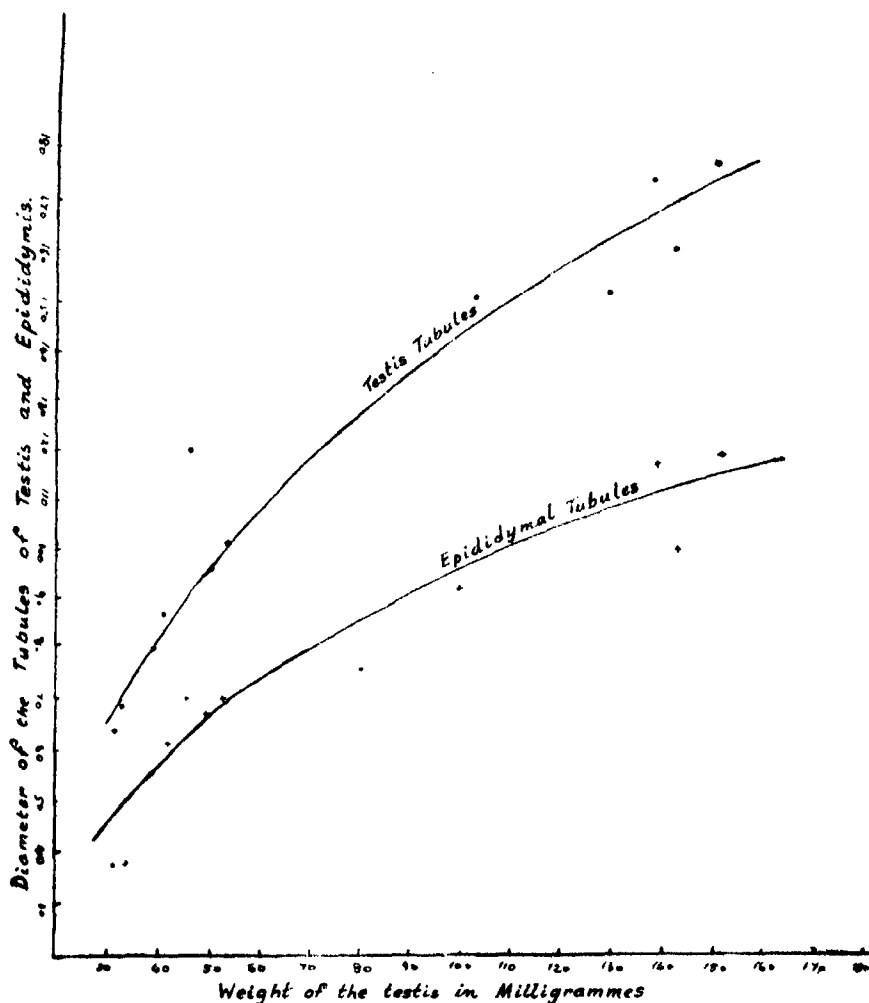
During January and February the diameter of the epididymal tubules reaches a maximum of  $114\mu$  to  $128\mu$  and all the lumina are packed with spermatozoa. The intertubular space is negligible (Plate VI, Fig. 5 and Plate VII, Fig 7).

Text-Fig. 5 is a graph to indicate the increase in the diameter of the epididymal tubules during December, January and February, and its comparatively narrow condition during the rest of the year.



TEXT-FIG. 5. Graph to indicate the increase in the diameter of the tubules of epididymis during the breeding season. The diameters are the largest between the months of January to March. The diameters are measured in microns.

Text-Fig. 6 shows the relationship that exists between the increase in the diameter of the tubules of the epididymis and the increase of the weight of the testis.



TEXT-FIG. 6. The diameters of the tubules of the testes and of epididymis plotted against the weights of the testes. The graph is a rising curve indicating that the diameters increase with the weight of the testes. The upper graph indicates the relation between the weight of the testes and the diameters of the testes tubules and the lower graph shows the relation between the weight of the testes and the diameter of the tubules of the epididymis.

The figures clearly show that the increase in the diameter of the epididymal tubules runs parallel to the increase in the weight of the testis, which in its turn is directly related to the functional activity of the testis.

#### CONCLUSIONS

1. *The Breeding Season.*—The progressive increase in the weight of the testes during the months from December to January, and its weight being at its lowest during the other months of the year taken along with the fact



that the testis contains the spermatozoa only during these months, indicates that the height of sexual activity is reached in these animals during these three months and the animals are sexually quiescent during the rest of the year.

The examination of the males alone cannot give us any definite proof regarding the exact period of copulation. The occurrence of large swarms of spermatozoa in the epididymis during January and February indicates the probability of copulation during these months. But the examination of the female genitalia (Gopalakrishna, 1947)<sup>a</sup> has revealed that the females have not only not ovulated on the 10th February but their genitalia contained no spermatozoa. Thus copulation had not occurred till the 10th of February. The conclusion therefore seems to be that the males become sexually active and functional nearly 6 to 8 weeks before the females ovulate. Further during the months of July and August the females are all lactating, and there is no evidence to show that lactating females become pregnant. Hence the absence of collection during July and August does not seriously impede us from concluding, without any possibility of error, that the males like the females experience an annual breeding cycle, and this roughly corresponds to the same months as the females, the males becoming sexually active a little (6 to 8 weeks) earlier than the females.

After copulation which I have shown to occur between the 10th February and the 22nd March (1947 *a*), the males probably live separately from the females, for during the rest of the summer though many expeditions were made and large number of specimens collected, the number of males captured was very low (Table I).

2. *Age of Maturity*.—A second important conclusion we may arrive at from the available data is that, as all the males collected during December, January and February were sexually active, animals born at about the end of June must have a rapid growth and must become sexually mature during the next January, *i.e.*, before they are one-year old. This seems to be a very unique phenomenon in the case of the insectivorous bats for no other author has recorded a similar feature in any of the microchiroptera. All authors are, however, agreed upon the fact that the bats have a very rapid period of growth even though they may remain sexually immature. But they believe that the bats come to sexual maturity during their second season.

Roland E. Miller (1939)<sup>7</sup> stated that "Young males of *Myotis lucifugus lucifugus* and *Myotis grisescens* do not enter into reproductive activity until their second spring". Harrison Matthews (1937)<sup>8</sup> working on the British Horse-shoe bats found that "The males do not reach their functional sexual activity until their second autumn".

And this view is confirmed by most of the authors working on the bats living in cold and temperate climates. This is probably because in these bats which inhabit the colder climates copulation takes place during autumn and the spermatozoa hibernate in the genital tract of the female during winter and fertilize the ova in the next spring. In the tropical bats on the other hand the problem of winter hibernation is bypassed and the males are nearly seven months old at the breeding season.

But Baker and Bird (1936)<sup>1</sup> working on the tropical bats in New-Hebrides stated that "None was definitely too small to be adult, but it is a well-known fact that bats become fully grown before they are sexually mature. The young could be detected by the size of their testes, which usually fell, fairly sharply into two groups as regards size. Those of the young naturally weighed about two milligrammes (both testes together), and sperms were never found in their epididymides".

Van der Stricht (1910) was the only other author who recorded that in *Nyctalus noctula*, in Europe, the young females copulate in the same year as that in which they are born (from Baker and Bird).

The observations recorded in this paper give ample evidence to show that *Scotophilus wroughtoni* becomes sexually mature before it is one year of age. All the specimens collected in January and February were fecund, as indicated not only by the testis-weight but also by their microscopic structure. It would indeed be highly improbable that all collections during these months should not include a single immature specimen—the greater probability is that all males and females do become sexually active in their first year. Probably the variation in the period of copulation makes all the difference between the bats of the temperate and the tropical zones regarding the age at which they attain their sexual maturity.

As regards the determination of the age in these male bats it is impossible to decide from their sexual activity only. I have only to reiterate the statement of Harrison Matthews (1937) "The changes taking place in their genitalia do not throw any light on the duration of life of these animals, though presumably it is approximately the same in both sexes".

A detailed study of the interstitial cells of the testis and the changes in the accessory reproductive organs in the male of this species will be dealt with in a separate paper.

#### SUMMARY

1. Twenty-nine male specimens of the bat *Scotophilus wroughtoni* were collected during the different months of the year. Collection were made during two successive years from May 1945 to April 1947.

2. The weight of the testis of these bats increases from the month of December and reaches a maximum weight during February. There is an increase nearly four times in the testis-weight from September to February.

3. The diameter of the seminiferous tubules also increases corresponding to the increase in the testis-weight and progressively vigorous spermatogenetic activity is seen in the germ cells. The diameter of the tubules of the epididymis also increases during these months.

The occurrence of sperms in the tubules of the testis and the epididymis indicates that the breeding season is confined to these months.

4. The males, like the females, probably attain their sexual maturity in their first season, i.e., before they are one year old.

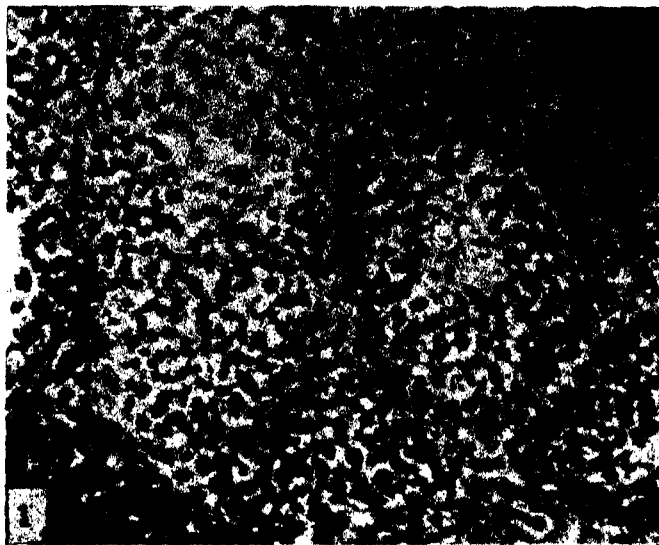
#### ACKNOWLEDGEMENT

I am deeply indebted to Prof. M. A. Moghe for valuable guidance and help throughout my work. My thanks are also due to Mr. P. A. Ramakrishna Iyer, M.Sc., Fellow of the National Institute of Science, Central College, Bangalore, for the technical guidance and training in the initial stages of my work.

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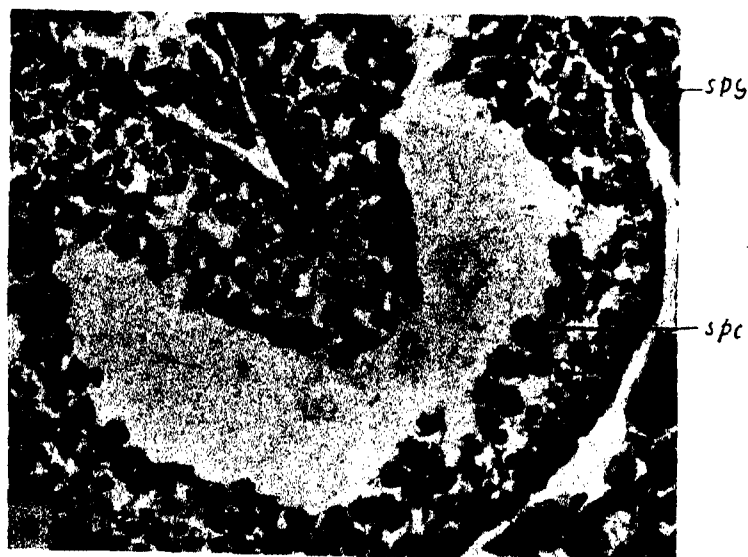
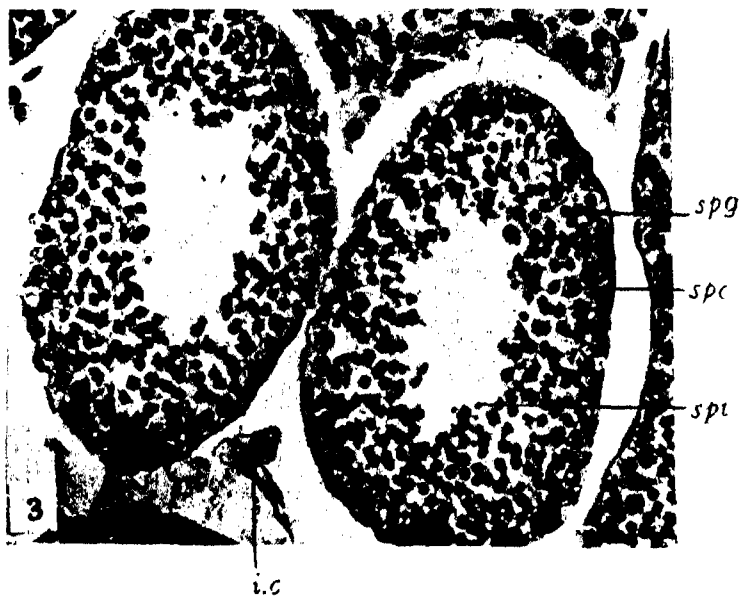
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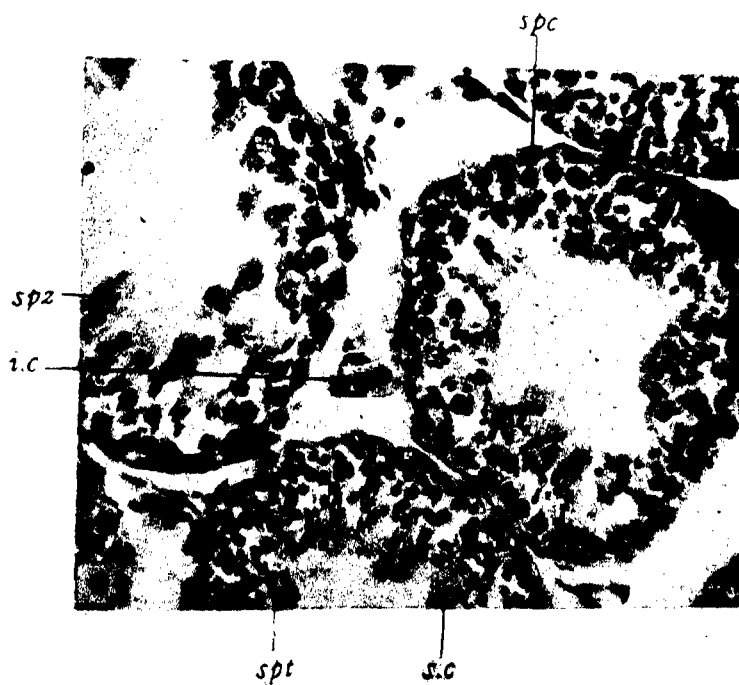
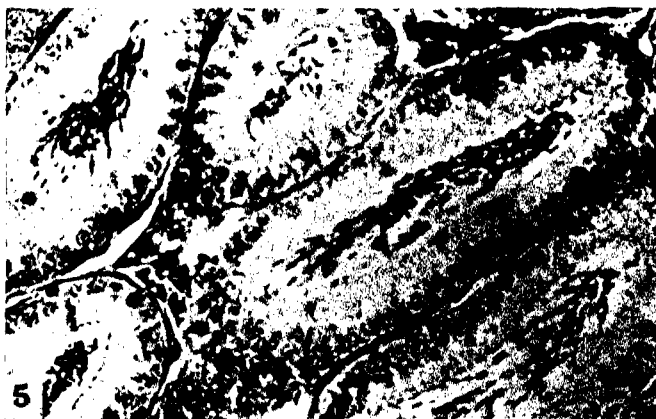
(P. S.—References marked in asterisk were not available in their original.)

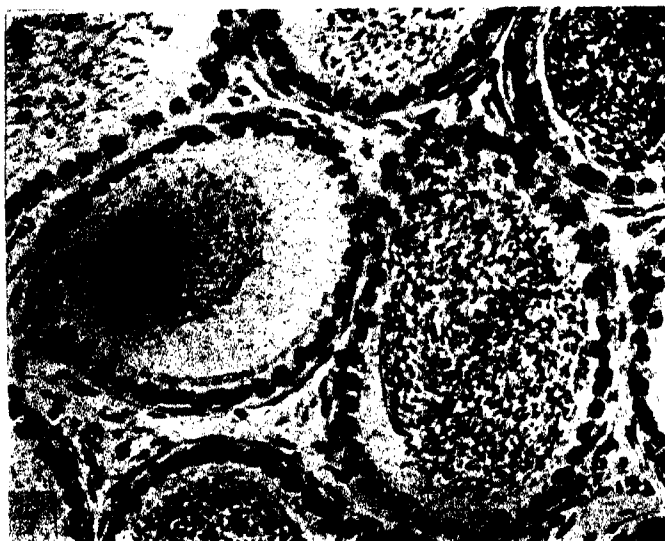


0.2 mm.









### EXPLANATION OF PLATES

All figures are photomicrographs. *Magnification of all figures is the same as of Fig. 1.*

FIGS. 1-7. Fig. 1. Two seminiferous tubules from a transverse section of the testis of a specimen collected in May (17-3-1945). The tubules have no lumen and consist of resting spermatogonial cells. Fig. 2. Epididymal tubules of the same specimen. The tubules are empty, i.e., contain no spermatozoa. There is a large amount of intertubular tissue. Fig. 3. Two seminiferous tubules from a transverse section of the testis of a specimen collected in December (7-12-1946). The cells are in various stages of spermatogenesis. The tubules have a distinct lumen. Fig. 4. One tubule from a transverse section of the testis of a specimen collected in January (8-1-47). Note the large lumen. Most of the germinal cells are in the final stage of spermatogenesis. The spermatocytes are abundant. Fig. 5. Epididymal tubules of the same specimen showing the sperms in the lumen of the tubules. The epithelium of the tubules is still columnar. The lumen is not so large as it is in a later specimen. Fig. 6. Seminiferous tubules from a transverse section of the testis collected in February (10-2-1946). Spermatids and the spermatozoa are abundant. Fig. 7. Epididymal tubules of the same specimen. The lumen is large and full of spermatozoa. The epithelial cells of the tubules are cubical instead of columnar.





# CTENOPLANA BENGALENSIS N. SP. FROM THE MADRAS PLANKTON

By C. P. GNANAMUTHU AND R. VELAPPAN NAIR

(From the University Zoological Research Laboratory, Madras)

Received March 24, 1947

(Communicated by Prof. S. G. Manavala Ramanujam, F.A.Sc.)

## INTRODUCTION

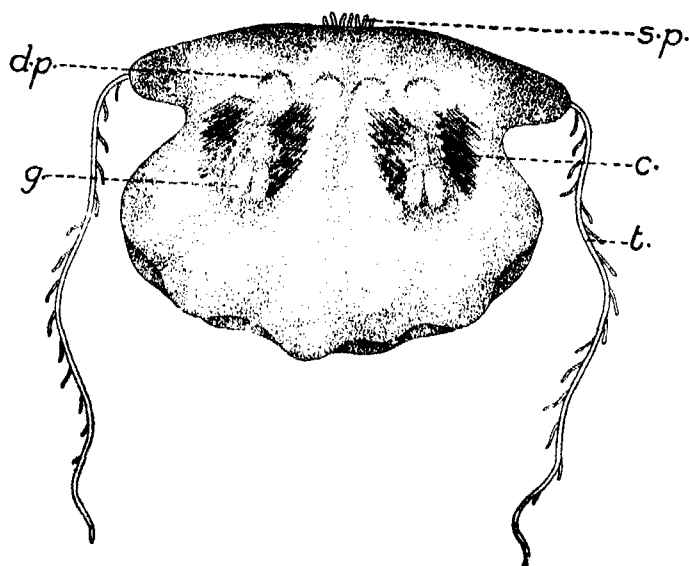
FROM the time of the discovery of the first species, *Ctenoplana kowalevskii* by Korotneff in 1886, Willey (1897) described *Ctenoplana rosacea* and *Ctenoplana korotneffi*, Dawydoff\* (1929 and 1936) recorded *Ctenoplana duboscqui* and *Ctenoplana perrieri* and Yoshi (1933) added *Ctenoplana maculomarginata* and *Ctenoplana muculosa*. Menon (1927) noted the occurrence of *Ctenoplana indica* in Madras, but as he did not publish any account of it, the *Ctenoplana* described in this paper is treated as a new species.

## GENERAL CHARACTERS

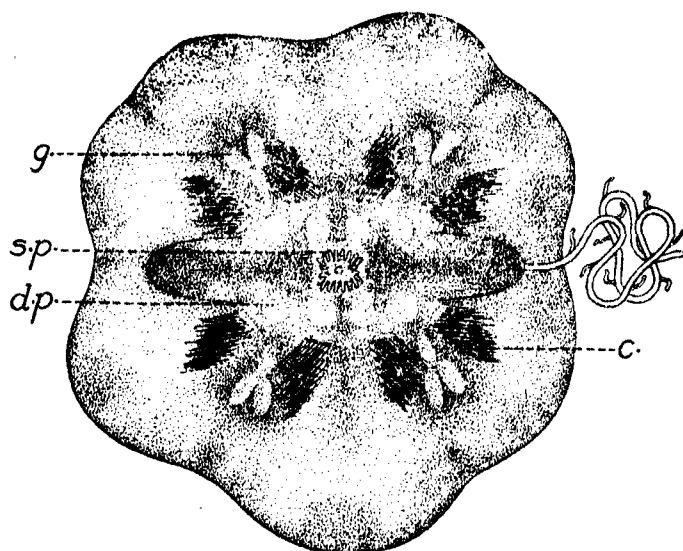
The specimen was found in the tow-net collection of the coastal plankton made off Madras on the 13th November 1946, and was studied alive in the laboratory for over a week. It endured rough handling while it was frequently pipetted from slide to dish and to the aquarium for purposes of observation and for changing the sea water. It was fed with live plankton and also with the flesh of prawn. The specimen measures 4 to 4.5 mm. and about 6 mm. when fully expanded. The tentacles when fully extended measure 15 mm. in length. While swimming, the lower or stomodæal part of the body appears distinct due to a constriction above it and then the oral edge is seen to be thrown into a number of lobes which come together and nearly close the mouth. In such a condition the body measures only 2 mm. in height and the form of the animal does not differ very much from that of the typical bell-shaped swimming ctenophore. While creeping, however, the mouth is opened out and the stomodæum is completely everted and extended all round the central region of the body which appears like a conical elevation much like a helmet. The outer margin of the flattened part is entire, devoid of the lobes seen when the animal is swimming. Nevertheless

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\* Dawydoff has also described *Planoctena agnia*, *Planoctena yuri* and *Planoctena esulleyi*.

TEXT-FIG. 1. Side view of *Ctenoplane bengalensis*

the entire shape of the conical as well as the flat portions of the body keep changing owing to the constant extensions and contractions in different directions.

TEXT-FIG. 2. Dorsal view of *Ctenoplane bengalensis*

The creature moves about by swimming and creeping on floating objects. Swimming is entirely ciliary and the animal keeps all the comb

plates of the eight costæ in quick motion and moves with its aboral pole forwards trailing the tentacles fully extended. Creeping or gliding over objects like a flat worm is effected by movements of portions of the flattened region of the body. This is, however, facilitated by the working of the comb plates of a few of the ribs. But when the animal is about to leave the substratum the comb plates of all the costæ are brought into play and the central thick region is elevated to its maximum height until finally it rises and swims upwards. Occasionally the animal floats on the surface of the water in a fully expanded condition. The method of ingestion of food particles is interesting. When given a small piece of prawn flesh, the animal approaches it mouth forwards lying on the side of the body. It widens its mouth and turns over covering the bit with the pharynx and erecting the aboral part of the body. If the flesh is given when the animal is creeping, it glides over the food and engulfs it. During the process of ingestion the stomodæum becomes completely everted and flattened.

The coloration is neither marked nor uniform. It is of a yellowish brown colour with darker brown patches. These patches which are scattered along the margin of the flattened part and crowded in the region of the tentacle sheaths and the gonads appear to be of cells of the nature of melanophores and have numerous slender and anastomosing extensions by the contraction of which the shape and intensity of the patches are altered. The gonads are yellowish white and stand out conspicuously. There are also white pigment spots scattered all over the body but these are not very marked or defined owing to the dull background coloration. The tentacles and their branches are colourless and transparent. The comb plates are iridescent. Though observed in the dark room the animal did not show any capacity for phosphorescence in spite of repeated provocative handling. The animal is very sensitive to bright light and energetically contracts changing its form frequently.

#### MORPHOLOGY

The eight costæ and their combs are very marked. Each rib is narrow and is flat at the aboral end while it tapers to a point orally. Each costa bears eight combs along its length. The width of the comb towards the oral end is narrower in accordance with the decreasing width of the rib, the last comb being extremely narrow bearing only a few cilia. The cilia are very long and are united up to about two-thirds of their length. The cilia of the upper combs are half as long as the costa itself. The cilia beat towards the mouth.

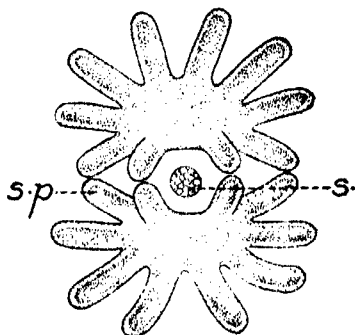
The statolith is small, spherical, opaque and granular in appearance. It is frequently hidden from view by the closing together of the finger-shaped

TABLE I

Species	Size	Colour	Shape	Sensory Papillae	Other Features	Locality
<i>C. korotneffi</i> Korotneff, 1886	7 mm. when moderately expanded. Height while swimming 3 mm.	Crimson with regular mahogany brown spots	Body in swimming attitude shaped like a truncated pyramid. Median dorsal surface concave. Free margin of skirt frilled	30-32 sensory papillae (18-20 external and 12 internal)		West coast of Sumatra and South Annam (Bay of Nhatrang)
<i>C. rosata</i> Willey, 1897	6 mm.	Crimson	Body in swimming attitude of a quadrilateral form. Median dorsal surface convex. Free margin of skirt plain	?		Eastern Archipelago of New Guinea
<i>C. korotneffi</i> Willey, 1897	6 mm.	Green	Body in swimming attitude roof-shaped. Median dorsal surface upraised into two upright end-knobs. Free margin of skirt slightly frilled	?		Eastern Archipelago of New Guinea
<i>C. duboquii</i> Dawydoff, 1929	6.5 mm. when fully expanded. Height while swimming 2.5 mm.	Grayish white with the central thick region olive green or intense yellow olive sprinkled with yellow dots on the peripheral region. Tentacular sheath orange brown		23 sensory papillae (15 external and 8 internal)	Costa with a single comb plate	South Annam (Bay of Nhatrang)
<i>C. maculomarginata</i> Yoshi, 1933	3 mm when moderately expanded and twice as large when fully extended	Pale yellowish green or light grayish olive with yellowish brown spots arranged at regular intervals along the margin		20-24 sensory papillae		Misaki

<i>C. mesoleuca</i> Yoshi, 1923	5-8 mm. when moderately expanded and twice as large when fully spread out	Light greenish yellow or clear pink with 13 or 14 yellowish brown spots having dark purple pigment particles arranged along the margin	24 sensory papillae	Misaki
<i>C. ferrieri</i> Dawydoff, 1926	6 mm. Height while swimming 2.5 mm.	Bright emerald green with scattered orange vermilion spots arranged in clusters. Tentacular sheaths shining vermilion	24 sensory papillae (14 external and 10 internal)	South Annam (Bay of Nha-trang)
<i>C. indica</i> Menon, 1927	4-4.5 mm. when moderately expanded and 6 mm. when fully expanded. Height while swimming 2 mm.	Yellowish brown with dark brown patches along the margin of the flattened portion. Regions of tentacular sheaths and gonads dark brown	20 sensory papillae	Madras (Bay of Bengal) Madras (Bay of Bengal)
<i>C. bengalensis</i> n.sp.				

sensory papillæ. There are twenty sensory papillæ. These papillæ are long and slender when extended and are capable of contraction and supple movements. The sensory region is elliptical with the semicircular ring of sensory papillæ bordering each side. The entire area containing the aboral sense organ, the polar fields and the sets of papillæ is frequently tucked in and thrust out especially when the animal is irritated. The papillæ and the polar fields are not pigmented.



TEXT-FIG. 3. The aboral sense organ

The two tentacles are long, highly contractile and bear uniseriate branches. These branches are regularly arranged, but decrease in length and number towards the distal extremity of the tentacle. The tentacle is extruded and withdrawn through the narrow mouth of the spacious tentacle sheath. Through the transparent sheath, when the pigmentation above it becomes lighter, the root of the tentacle can be seen at the proximal end of the sheath.

The gastrovascular system begins with the wide stomodæum or pharynx, the eversion of which is responsible for the planarian appearance of the creature. When not everted the stomodæum forms nearly half the height of the animal. When the animal is allowed to attach to a coverslip, an oral view into the interior of the œsophagus can be obtained. The wall of the œsophagus which is very short is produced inwards into a number of irregular highly contractile branching folds. Beyond the œsophagus, when it is widened, can be seen the stomach and the peripheral canals. Of these canals there are six on each side, those in the tentacular plane being wider. The canals branch repeatedly till they merge into the network of canals in the flattened part of the body. The stomach is spacious and is elongated along the tentacular axis. When the animal contracts its body eight sac-like projections, the dorsal papillæ, can be seen pushed out on the aboral side of the body. The anal pores and the anal canals could not be observed owing to the ramifying pigmentation.

The gonads are four in number situated in the four interradii between the tentacular and stomodæal axes.

#### GENERAL REMARKS

The important taxonomic features of all the known species of *Ctenoplana* as can be gathered from a complete search of the available literature is given in Table I. A comparison of the characters of the Madras form with those of the other species of *Ctenoplana* shows clearly that the species described here is new to Science. In the number and arrangement of the sensory papillæ *Ctenoplana bengalensis* differs from all the known species. The sensory papillæ of *Ctenoplana kowalevskii*, *Ctenoplana duboscqui* and *Ctenoplana perrieri* are arranged in two sets, one internal and the other external. The arrangement of these papillæ in *Ctenoplana maculomarginata* and *Ctenoplana muculosa* appears to conform with that of *Ctenoplana bengalensis*, but there is variation in the number of the sensory papillæ. There is no information about the sensory papillæ of *Ctenoplana rosacea* and *Ctenoplana korotneffi*. Similarly the coloration of *Ctenoplana bengalensis* is markedly different from that of all the recorded species of *Ctenoplana*.

It is significant that this species also like all the known species of *Ctenoplana* has been collected from the plankton. *Ctenoplana kowalevskii* was found drifting in a current of water along with numerous *Porpita*. *Ctenoplana rosacea* and *Ctenoplana korotneffi* were taken from a cuttle bone which was floating and moving along the current. *Ctenoplana duboscqui* and *Ctenoplana perrieri* were collected from the plankton while *Ctenoplana maculomarginata* and *Ctenoplana muculosa* were obtained from floating sea weeds. From these recorded observations it is evident that *Ctenoplana* is essentially a pelagic form like the majority of the ctenophores, both during the swimming and creeping conditions, and inhabits the coastal waters. Mention may be made here that all the recorded species of *Planoctena* were also collected from the plankton except *Planoctena caulleryi* which was collected from an Octocorallian belonging to the genus *Xenia*.

#### ACKNOWLEDGEMENTS

Our thanks are due to Mr. C. Vedachalam for bringing the specimen to our notice and to Mr. S. Gopalan Nair for translating the French articles. We are also thankful to Dr. S. G. M. Ramanujam for lending us a few journals.



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## EXPLANATION OF PHOTOGRAPHS

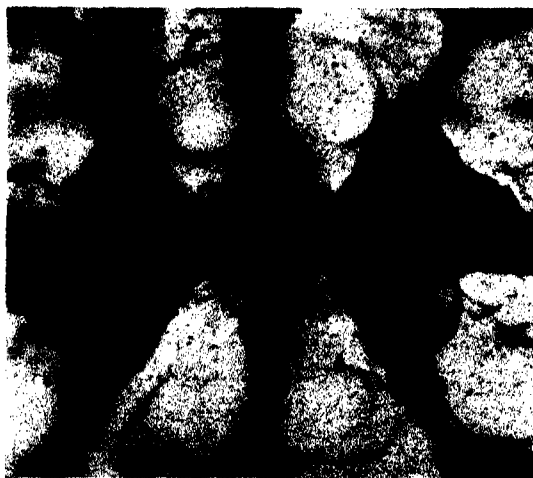
- Photomicrograph 1. Dorsal view of *Ctenoplana bengalensis*.
- „ 2. The aboral region showing the sense organ, sensory papillæ and dorsal papillæ.

## KEY TO LETTERING

c.	costa.	s.	statolith.
d.p.	dorsal papilla.	s.p.	sensory papilla.
g.	gonad.	t.	tentacle.



1



2



## SOUTH INDIAN PHYCOMYCETES—I\*

*Pythium indicum* Sp. nov. causing a Fruit Rot of  
*Hibiscus esculentus* Linn.

BY M. S. BALAKRISHNAN, M.Sc.

Received August 11, 1947

(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

### INTRODUCTORY

IN November 1946, the writer received a few diseased fruits of *Hibiscus esculentus* L. collected by Mr. C. L. Sundararajan, B.Sc. (AG.), from his estate at Podanur. It was stated that these specimens were representative of a type of decay responsible for considerable damage. Incidence of fruit rot was high during the months of October and November when there was very heavy rainfall with few sunny days and the reduction in yield amounted to over 50 per cent. The affected fruits showed large water-soaked, sunken, brownish lesions covered by a slight growth of fluffy white aerial mycelium. The tissues inside were found decayed and greatly softened with the result that the material gave a peculiar marshy odour. Microscopic examination revealed that the aerial mycelium consisted of non-septate hyphæ; moreover, the numerous oogonia with oospores in all stages of development and the accompanying antheridia found in portions of the aerial mycelium matted in severely rotted portions of fruits, indicated that the pathogen was in all likelihood a species of *Pythium*. This was confirmed when bits of aerial mycelium left overnight in sterile distilled water or soil leachate produced sporangia, their mode of formation and germination proving beyond doubt that it was a species of *Pythium* with filamentous sporangia. A survey of literature showed that no species of *Pythium* with filamentous sporangia has so far been recorded on this host. Further, this fungus also showed several interesting features and, therefore, a detailed study of the organism was undertaken.

### THE CAUSAL FUNGUS

Detailed examination of diseased tissues showed that the pathogen was intracellular; the softened decaying tissue was occupied by branched

\* Contribution from the I.C.A.R. School of Mycology, Agricultural Research Institute, Coimbatore.

non-septate hyphæ showing no definite orientation. At points where the hyphæ pierced the cross walls, they were markedly constricted. Pure cultures of the fungus were easily obtained by placing surface-sterilized bits of diseased tissue on oatmeal agar plates and transferring portions of the mycelium from the margin of the resultant growth to sterile media in tubes. Raper's technique was adopted to free the cultures from bacteria.

The fungus grew well on most of the agar media, growth being particularly luxuriant on oatmeal, French-bean and carrot agars. Of these three, oatmeal agar was found best and subsequent pure cultures were maintained on this medium. Growth was very rapid on most of the media at laboratory temperature (26–27° C.) a 10 cm. Petri dish being completely covered with dense aerial mycelium in 36 hours. Aerial mycelium was less luxuriant on synthetic media such as Raulin's agar or plain water agar. At laboratory temperature this isolate grew faster than fresh isolates of *P. aphanidermatum* (Eds.) Fitz. and *P. graminicolum* Subr. indicating that this must be one of the fastest growing tropical species of the genus known.

**Morphology.**—The hyphæ varied from 4 to 12  $\mu$  in diameter, mostly 8 to 10  $\mu$ , and were profusely branched. The axial hyphæ were very stout, often measuring over 10  $\mu$  in diameter, while the lateral branches were comparatively slender, measuring 4 to 6  $\mu$  in diameter. Clavate and falcate appressoria, either simple or compound, were formed in abundance wherever the hyphæ came into contact with obstruction, *i.e.*, the side or bottoms of a Petri dish or the side of a test-tube. These appressoria were considerably broader than the parent hyphæ often exceeding 20  $\mu$  in thickness, and were filled with dense granular protoplasm (Fig. 1, A, B). The hyphæ were for the most part non-septate, becoming septate only in the older portions of the intra-matrical mycelium and were filled with homogeneous finely granular protoplasm without any inclusions or vacuoles.

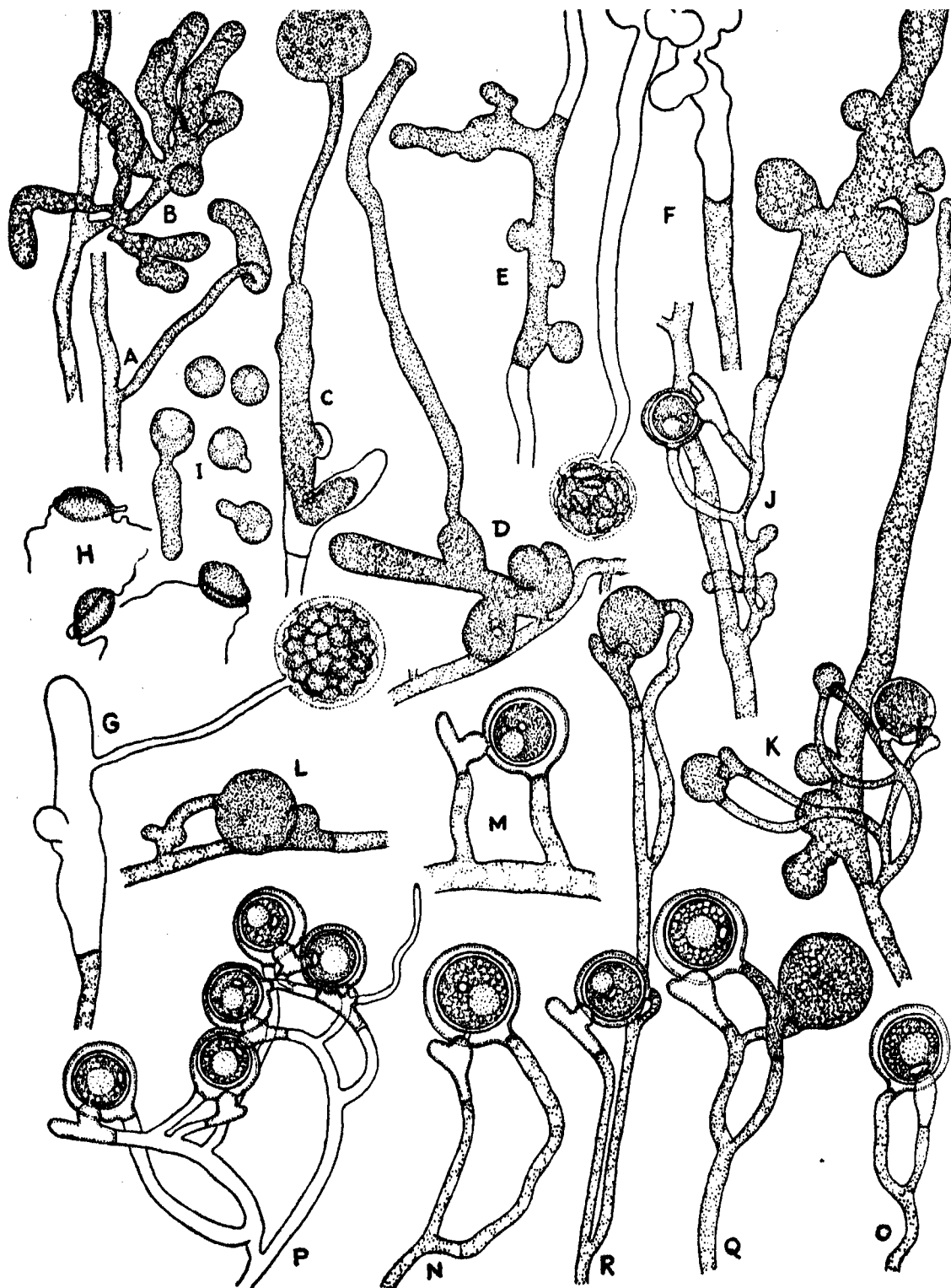
Asexual reproduction was easily induced by excising small bits from an oatmeal agar-plate culture of moderate thickness and transferring them to a shallow layer of sterile distilled water or steamed soil leachate in a sterile Petri dish. In such preparations, abundant sporangia were produced in 12 to 15 hours. Experience showed that the production of sporangia was more rapid in soil leachate than in distilled water; also, more sporangia germinated indirectly (by producing zoospores) in soil leachate than in distilled water.

The sporangia were inflated structures, rarely lobed and digitate but never forming such intricate complexes as in *P. aphanidermatum*. They were always thicker than the parent hypha, ranging from 15 to 20  $\mu$  in diameter

and 100 to 250  $\mu$  in length and were either terminal or intercalary. The sporangia opened by an evacuation tube of varying length, either subterminal, terminal or lateral in position (Fig. 1, C, D, E, F and G), usually as long or longer than the sporangium, occasionally shorter, but never very short. Delimitation of the zoospores took place within a vesicle formed at the tip of the evacuation tube which was slightly wider at the tip of dehiscence than at the point of its origin. The number of zoospores formed within a single vesicle varied from 25 to 150 depending on the size of the sporangium. The zoospores actively swarmed within the vesicle for about a minute, being released by its rupture. They were reniform, with a prominent groove or hilum and possessed two equal cilia inserted on the groove (Fig. 1, H). When free swimming the zoospores measured 12 to 15  $\mu$  by 10  $\mu$  and when encysted 10  $\mu$  in diameter. They always germinated by the production of 1 to 3 germ tubes (Fig. 1, I). No case of repeated emergence was seen. Under unfavourable conditions the sporangia germinated directly, producing one to several germ tubes. ✕

On oatmeal and French-bean agars the fungus promptly developed sexual bodies in abundance. The first formed oogonia were always terminal on short lateral branches. These oogonia were accompanied by typically monoclinal antheridia which originated a short distance below the oogonium and were borne terminally on a straight stalk. In shape, the antheridia were filamentous-clavate, only slightly thicker than the stalk from which they were cut off. Very often, the antheridia possessed a distal lobe giving them a characteristic appearance. Though most of the first formed antheridia were terminal in position, they were also sometimes intercalary, the distal segment of the antheridial stalk usually remaining short at least up to the time of fertilization (Fig. 2, G). The most characteristic feature of the sexual apparatus in this fungus, however, was the pronounced curvature of the oogonial stalk towards the antheridium borne on a straight stalk (Fig. 1, J, K, N, O, Q, R). This feature is shared by this species with two other species of *Pythium*—*P. indigofera* Butler and *P. deliense* Meurs. This phenomenon is in sharp contrast to the behaviour in other species of *Pythium* in which the converse is the rule.

As the cultures grew older, more sexual bodies were formed by the lateral branch which produced the primary sexual apparatus, these being often formed as a result of further growth of the stalks of the primary antheridium and oogonium, so that often 5–10 oogonia and their antheridia were borne together in a closely knit cluster on the fertile lateral giving a characteristic habit to the mycelium. When such secondary sexual bodies were produced, the secondary oogonium usually took its origin from the



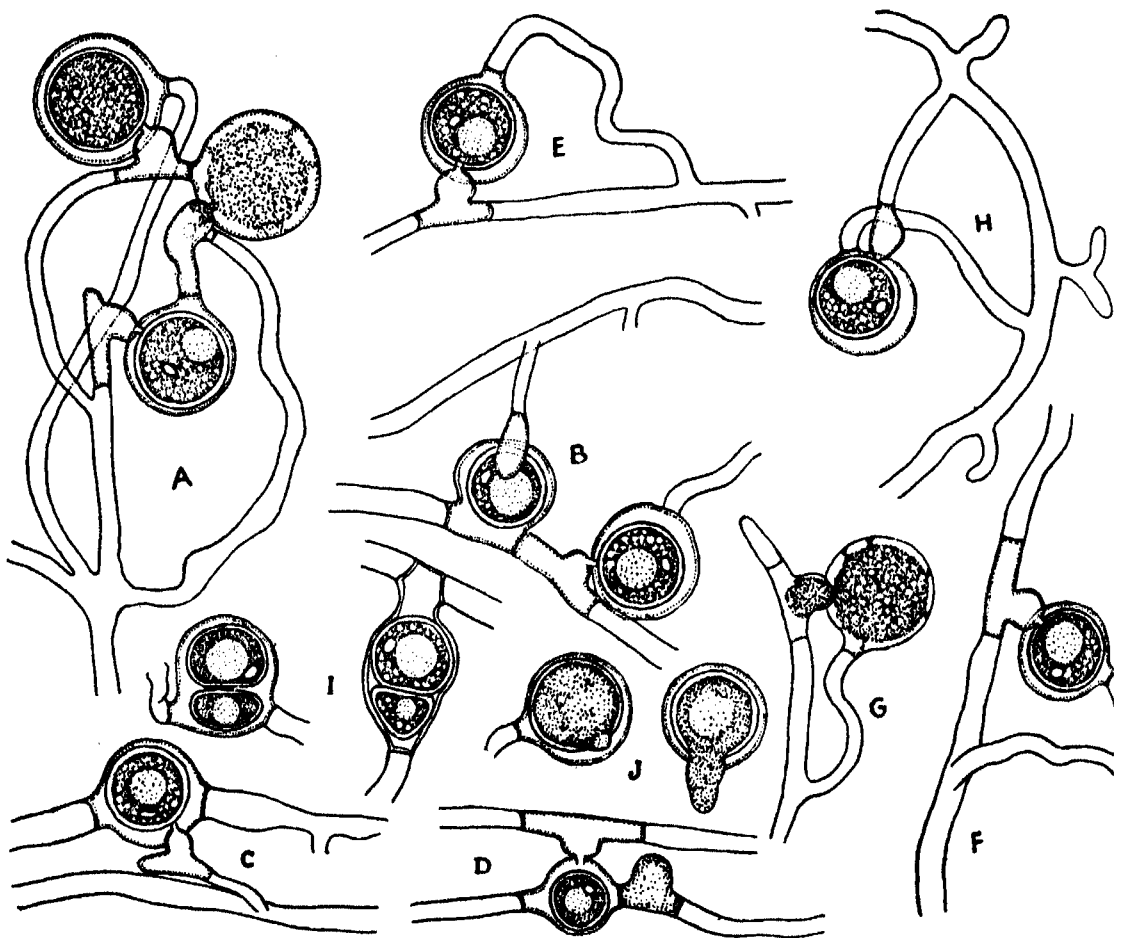
TEXT-FIG. 1. Sexual and asexual reproductive structures of *Pythium indicum*. The magnification is  $\times 480$  in the case of figs. A-G, J, K, P and R and  $\times 680$  in the case of figs. R, I, L, M, N, O and Q.

primary antheridial stalk and was fertilized by an intercalary antheridium formed on the stalk of the primary oogonium adjacent to it. This process was repeated till finally a cluster of sexual bodies was formed round the primary sexual apparatus (Fig. 1, *P, Q*, Fig. 2, *A*). Exceptions to this general rule were also found; sometimes the primary antheridial stalk branched to form another antheridium while the primary oogonial stalk produced an oogonium and so on (Fig. 1, *R*). In all cases there finally results a cluster of sexual organs borne on a single fertile lateral. As far as the writer is aware, such a development of sexual bodies giving rise to clusters has not been observed in any other species of *Pythium*.

Again, though the first formed oogonia were always terminal accompanied by strictly monoclinal antheridia, considerable diversity in the shape, position and relationship of the antheridium to the oogonium was seen in the later formed sexual bodies. As already stated the typical sexual apparatus in this form consisted of a terminal oogonium which was strongly curved towards the monoclinal antheridium borne terminally on a straight stalk. Sometimes, however, this relationship was not seen, the antheridium and oogonium being borne on parallel branches without any curving of the oogonial stalk towards the antheridium (Fig. 1, *M*). Another variation commonly seen was that the oogonium was intercalary, sub-spherical or barrel-shaped, fertilized by a diclinal antheridium which in its turn may be either terminal or intercalary (Fig. 2, *B, C, D*). Or, the antheridium may be intercalary on the parent hypha itself, the oogonium being brought into contact with it by a pronounced curvature of the oogonial stalk (Fig. 2, *E, F*). Such a diversity of antheridial relationship is not seen in the two congeneric forms mentioned above—*P. indigofera* and *P. deliense*.

The oogonia varied in diameter from 12 to 24  $\mu$  (av. 19.6  $\mu$ ) and were usually accompanied by only one antheridium. After fertilization an oospore was formed inside the oogonium showing the internal organization characteristic of the ripe oospores of most species of *Pythium*, a single reserve globule surrounded by densely granular protoplasm in which was imbedded a spherical or kidney-shaped refringent body. Developmental irregularity sometimes led to the formation of two oospores within a single oogonium (Fig. 2, *I*). The oospores were aplerotic with a moderately thick wall and varied in diameter from 10 to 20  $\mu$  (av. 15.94  $\mu$ ) and germinated by the production of a germ tube (Fig. 2, *J*). Though the sexual bodies were borne separately from the sporangia, they were often found associated with sporangia as reported by Butler for his *P. indigofera*, a congeneric form. (Fig. 1, *J, K*).





TEXT-FIG. 2. Sexual reproductive structures of *P. indicum*.  $\times 680$ .

**Cultural.**—The growth of the fungus was studied on the following culture media used as agar slants in test-tubes in comparison with a type culture of *P. indigofera* obtained from the National Collection of Type Cultures, Delhi, and a fresh isolate of *P. aphanidermatum* isolated by the writer from rotting tomato fruits collected at Coimbatore. There were three replications for each medium used and growth was compared after 15 and 22 days at 20° C. The results of these studies are given in the table below:

It will be seen from the above table that the *Pythium* isolated from *H. esculentus* is the most vigorous grower of the four congeneric species compared here. *P. aphanidermatum* also shows abundant aerial mycelium

TABLE I

Comparison of growth of *P. indigofera*, *P. aphanidermatum*, *P. deliense*\*  
and the *Pythium* isolated from *Hibiscus esculentus*

Culture medium used	<i>P. aphanidermatum</i>	<i>P. indigofera</i>	<i>P. deliense</i>	<i>P. sp. (Hibiscus esculentus)</i>
1. Oatmeal agar ..	Abundant cottony aerial mycelium filling the entire tube flush with the top of agar slant	Meagre aerial mycelium forming a mat on the surface of agar-growth mostly submerged	Moderate aerial mycelium, thin in upper half, dense in lower half though not filling it	Abundant cottony aerial mycelium filling whole tube; sharp horizontal demarcation flush with top of agar slant appearing as a solid plug
2. French bean agar ..	do	do	..	do
3. Carrot agar ..	Abundant aerial mycelium though not so luxuriant as in oats agar; filling tube with nearly horizontal demarcation	Growth poorer than in oatmeal or frenchbean agars. Meagre aerial mycelium, growth submerged	Moderate aerial mycelium, thin in upper half, more dense in lower half	do
4. Nutrient agar ..	Moderate aerial mycelium filling half of tube, forming a dense mat on the surface of the slant	Very poor growth, entirely submerged, no aerial mycelium	..	Moderate aerial mycelium, more abundant than <i>P. aphanidermatum</i> in this medium forming a thick mat on the surface of the agar slant
5. Raulin's agar ..	Growth poorer than in nutrient agar, moderate aerial mycelium matted on the surface	Practically no growth even after one month	Extremely meagre aerial mycelium. More dense in upper than in lower half	do
6. Water agar ..	do	Very poor growth; no aerial mycelium	..	do

\* Note.—The data given for *P. deliense* are taken from Meurs' account of the fungus.

TABLE II  
Inoculation Experiments with the *Pythium* isolated from  
*Hibiscus esculentus*

Plant used for the experimental	Parts inoculated	Result	Remarks
1. <i>Nicotiana tabacum</i> L.	Terminal bud, stem and leaves (unwounded)	-	
2. do ..	do (wounded)	+	Large brownish lesions were seen on the second day after inoculation. These spread rapidly. Aerial mycelium was seen on the affected parts on the 4th day after inoculation
3. <i>N. glutinosa</i> L.	Terminal bud, stem and leaves (unwounded)	-	
4. do ..	do (wounded)	+	Symptoms same as in <i>N. tabacum</i>
5. <i>Solanum melongena</i> L.	Stem, terminal bud and young leaves	+	Dieback was seen on the fourth day after inoculation. Only young portions of stem and young leaves were infected
6. do ..	Fruits (unwounded)	-	
7. do ..	do (wounded)	+	Sunken water-soaked lesions appeared round the point of inoculation on 2nd day and spread rapidly. The whole fruit was rotted in 7 days. Profuse aerial mycelium was seen on the fruit after the 4th day
8. <i>S. nigrum</i> L. ..	Terminal bud	+	Severe dieback was seen on the 3rd day after inoculation. The plant was killed in 10 days
9. <i>Capsicum annuum</i> L.	do	+	do
10. <i>Lycopersicon esculentum</i> Mill	do	+	Dieback not so severe as in <i>S. nigrum</i> and <i>C. annuum</i> . The plant was killed in 15-18 days
11. do ..	Fruits (unwounded)	+	Brownish lesions seen round the point of inoculation on 2nd day; fruits completely rotted in 5-7 days
12. do ..	do (wounded)	+	Infection more rapid than in unwounded fruits. Fruits were completely rotted in 4 days
13. <i>Datura fastuosa</i> L.	Terminal bud	-	
14. <i>Petunia</i> sp. ..	do	+	Severe dieback was seen. The plants were killed in 7-10 days
15. <i>Carica papaya</i> L.	Fruits (unwounded)	+	Fruits were completely rotted in a week
16. do ..	do (wounded)	+	Infection more rapid. Fruits were completely rotted in 5 days
17. <i>Cucurbita maxima</i> Duchesne ..	do (unwounded)	+	Infection more severe than in all other fruits tried. Even large fruits were completely rotted within a week

TABLE II—Contd.

Plant used for the experiments	Parts inoculated	Result	Remarks
18. <i>Cucurbita maxima</i> Duchesne ..	Fruits (wounded)	+	Fruits were completely rotted in 4 days. Abundant aerial mycelium similar to that described by Drechsler (1925) for cottony leak of cucumbers
19. <i>Zea mays</i> L. ..	Base of stem (wounded)	+	Severe stalk rot was evident three days after inoculation. The plants fell over and died in a week
20. <i>Pennisetum typhoides</i> Rich.	do	-	
21. <i>Amaranthus gangeticus</i> L.	Terminal bud	+	Infection severe. Dieback resulted and the plants were killed in 4-5 days
22. <i>Vigna catjang</i> Endl.	do	+	Infection slight confined to younger portions alone
23. <i>Hibiscus esculentus</i> L.	do (Seedlings in 2-leaved stages)	+	Both cotyledonary leaves drooped six hours after inoculation. Rotting was evident in 12 hours and rot spread rapidly downwards, killing the plant in 72 hours. Profuse aerial mycelium was seen on rotted parts.
24. do ..	do (seedlings in 4- and 6-leaved stages)	+	Infection evident 15 hours after inoculation. Dieback very severe, plants being killed within 5 days
25. do ..	Axils of leaves of young plants (8-leaved)	+	The petioles and laminae took infection and rotted in 36 hours. A lesion about 1" in diameter was seen at the node. No further spread of infection up or down the stem was seen
26. do ..	Fruits (wounded)	+	Infection very rapid, fruits being completely rotted in 3 days
27. do ..	do (unwounded)	+	do

though to a slightly lesser degree. According to Meurs (1934) the aerial mycelium of *P. deliense* is less luxuriant than that of *P. aphanidermatum*. *P. indigofera* is the poorest grower, presenting a marked difference in cultural behaviour from the other three species.

**Pathogenicity:** Numerous inoculation experiments were carried out to test the pathogenicity of this species and also to get an idea of the host range. These experiments were carried out on potted plants one to two months old except in cases where it is stated otherwise. The plants were well washed with sterile distilled water before inoculation and kept covered with bell jars after inoculation so as to maintain a high level of humidity. Fruits to be inoculated were thoroughly washed in an aqueous solution of mercuric

chloride (1 in 1,000) and several changes of sterile distilled water and kept in sterile moist chambers. In all cases plants and fruits similarly treated and kept under the same conditions without the inoculum were kept as controls. These remained healthy and unaffected throughout.

The results indicate that this species is likely to have a very wide host range. Only one species of *Pythium*—*P. de Baryanum* Hesse—has been recorded on *H. esculentus* so far (Ramos, 1926).

*Taxonomy.*—As stated already, the present species shows a close relationship to *P. indigofera* and *P. deliense* in the bending of the oogonial stalk and appended oogonium towards the antheridium. The antheridial stalk always remains straight. This phenomenon is in sharp contrast to the behaviour in other species of *Pythium* where the converse is the rule. However, it also exhibits certain differences from both the abovementioned species. From *P. indigofera* it can be easily differentiated by its more vigorous and robust growth on most agar media and especially on synthetic media like Raulin's agar in which *P. indigofera* is unable to grow at all. Moreover, in *P. indigofera* the oogonia are always terminal whereas in the form under discussion they are frequently intercalary; also, intercalary antheridia as met with in the present form are not seen in *P. indigofera*. Further, though much stress cannot be laid on the fact that the *Pythium* on *H. esculentus* produces abundant sporangia as against the rare occurrence of these structures in *P. indigofera*, it is useful as an additional distinguishing feature between the two species as also the fact that in this species the evacuation tube is as long or longer than the sporangium, never very short as described by Butler for *P. indigofera*.

From *P. deliense* which comes nearest to it the *Pythium* on *H. esculentus* differs in the production of abundant aerial mycelium on most culture media as opposed to the 'moderate' aerial mycelium of *P. deliense*. This difference is most marked in synthetic media as Raulin's agar. Meurs (*loc. cit.*), who first described *P. deliense* from tobacco in Sumatra says that it did not grow well in Raulin's agar and produced meagre aerial mycelium. The present form, however, produces fairly luxuriant aerial mycelium on Raulin's agar though not quite as abundantly as in richer media. Further, in this species, the sexual bodies and sporangia occur together, a feature which is absent in *P. deliense*, where sporangia and sexual organs occur separately. This feature is so characteristic of *P. deliense* that both Meurs and Middleton (1943) state it to be diagnostic for that species. The production of appressoria in abundance is another point of difference between these two species. Meurs says that appressoria are conspicuously absent in *P. deliense*. Finally,

the evacuation tube in the *Pythium* on *H. esculentus* is never shorter than the sporangium; in *P. deliense* on the other hand, it is often very short.

This fungus is also closely allied to *P. aphanidermatum*. The oogonia are similar in size and position and in instances when the oogonial stalk is not bent towards the antheridium as in cases of both sexual organs arising on parallel branches of the same parent hypha, or when the antheridium is diclinous, some difficulty may be experienced in distinguishing the two species, especially if the antheridium is intercalary as sometimes happens in this species. However, though intercalary antheridia are common to both species, they are more frequent in *P. aphanidermatum* and are typical of that species. Further, the antheridia in *P. aphanidermatum* are typically diclinous and intercalary and only occasionally monoclinal and terminal. As stated previously, the first formed antheridia in the present form, however, are typically terminal and monoclinal and are a characteristic feature of the fungus; intercalary and diclinous antheridia are atypical and infrequent. This feature can be taken as a very stable and satisfactory criterion for the differentiation of these two species. Finally, the *Pythium* on *H. esculentus* is further distinguished from *P. aphanidermatum* by the characteristic clustering of the sexual organs on the fertile lateral, the smaller size of the oogonia and oospores,—(oogonia  $27\mu$  and oospores  $26.55\mu$  in *P. aphanidermatum* as against  $19.6\mu$  to  $15.94\mu$  in this species), the less complex nature of the sporangia and the typical curvature of the oogonial stalk towards the antheridium which is always borne on a straight stalk.

The differences mentioned above are, in the writer's opinion, sufficient to consider this fungus distinct from *P. indigofera*, *P. deliense* and *P. aphanidermatum*. It is, therefore, presented as a new species, *Pythium indicum*.

*Pythium indicum* sp. nov.: Hyphae non-septate when young, irregularly septate when old, measuring  $4-12\mu$ , mostly  $8-10\mu$ , in diameter, sporangia terminal, infrequently intercalary, inflated filamentous, up to  $250\mu$  long, wider than the parent hypha, provided with lateral lobes though never forming intricate complexes. Zoospores 25 to 150, reniform, laterally biciliate, measuring  $12-15\mu$  by  $10\mu$  while free swimming and  $10-12\mu$  in diameter when encysted, monoplanetic, germinating by the production of 1-3 germ tubes. Zoospores delimited within a vesicle formed at the tip of an evacuation tube of varying length, as long or longer than the sporangium, never very short, with an enlarged tip of dehiscence; evacuation tube usually terminal though at times sub-terminal or intercalary.

Oogonia spherical, sub-spherical or barrel-shaped, terminal or intercalary, smooth walled, measuring 12 to  $24\mu$  (av.  $19.6\mu$ ) in diameter.

Antheridia typically monoclinal, rarely diclinal, single, the antheridial stalk usually straight with the oogonial stalk bent towards it. Rarely the antheridial and oogonial stalks may be parallel branches of the same parent hypha. Antheridial cell filamentous-clavate, terminal, rarely intercalary, often with a distal lobe, making apical contact with the oogonial wall. Fertilization tube moderately thick and clearly visible. Oospores smooth, aplerotic, with a moderately thick wall containing a single reserve globule and a single refringent body. Oospores measuring  $10.20\mu$  (av.  $15.94\mu$ ) in diameter, germinating by a germ tube. \*

Causing fruit rot of *Hibiscus esculentus* L. Podanur, Coimbatore, South India, collected by C. L. Sundararajan.

Type culture deposited in the Government Mycologist's collection of stock cultures, Agricultural Research Institute, Lawley Road P.O., Coimbatore, South India.

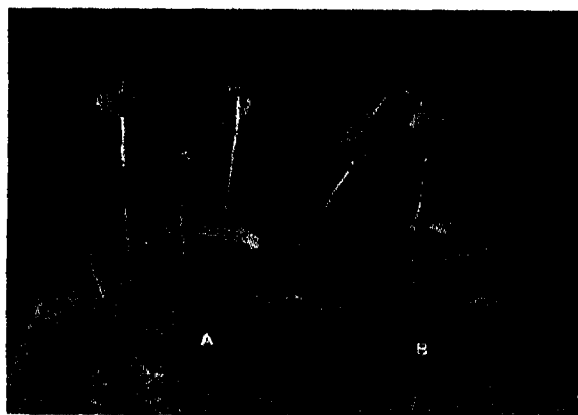
*Pythium indicum* sp. nov.—Hyphæ juvenes non-septate, veteres irregulariter septatae,  $4-12\mu$ , plurimum  $8-10\mu$  diam.; sporangia terminalis, rare interposita, inflatis-filamentosa, ramosissima, ca.  $250\mu$  longa, latora quam hypha parens, tubulis vel zoosporis germinantia; zoosporia reniformis, biciliatis,  $12-15 \times 10\mu$  diam. natantia,  $10-12\mu$  cystidiosacta, monoplaneticis, germinantibus uno vel tribus tubulis; oogonia terminalia, rare interposita, globosa vel sub-globosa,  $12-24\mu$  (av.  $19.6\mu$ ) diam.; antheridia monoclina, rare diclina, solitaria, pedicilli recti, pedicellus oogoniae curvatus ad antheridium, interdum pedicilli antheridium oogoniae paralleli; oosporae apleroticae, cuticulis mediocriter crassa, unicum globulum et corpus nicans habentes,  $10-20\mu$  (av.  $15.94\mu$ ) diam, tubulis germinantes.

Causat putredinem fructus *Hibisci esculentis*, Podanur, Coimbatore, South India. Leg. C. L. Sundararajan.

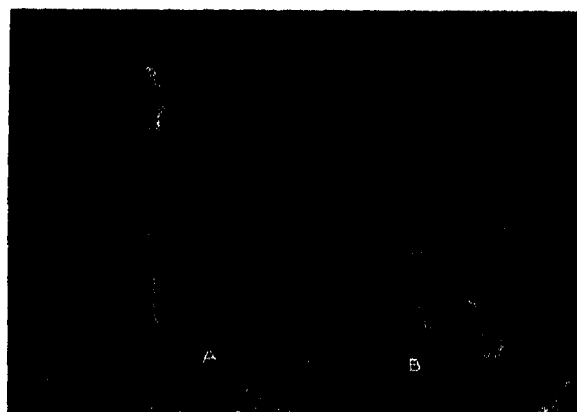
#### ACKNOWLEDGEMENTS

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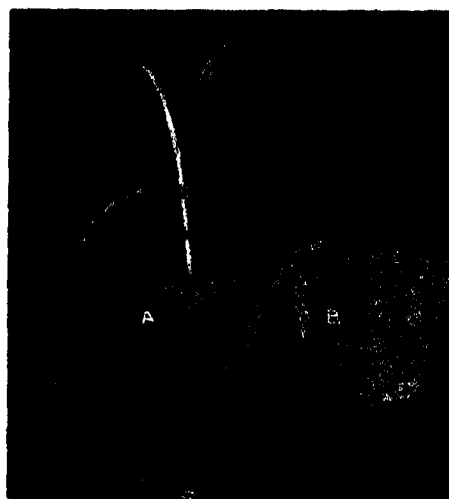
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#### INOCULATION TRIALS WITH *PYTHIUM INDICUM* SP. NOV.

FIG. 1. Inoculated (A) and healthy (B) seedlings of *Hibiscus esculentus*.

Note.--In (A) the two seedlings in the foreground show die-back due to inoculation on the terminal bud ; in the case of the two seedlings in the background inoculation was in the axils of the two lowest leaves which alone have rotted.

FIG. 2. Healthy (A) and inoculated (B) plants of *Petunia* sp. Note the dieback ; both plants were of equal height when inoculated.

FIG. 3. Fruits of *Cucurbita maxima* Duch. (A), wounded inoculated, (B), control and (C), unwounded inoculated. Photograph taken 5 days after inoculation.

FIG. 4. Healthy (A) and inoculated (B) plants of *Zea mays* L. showing stalk rot and falling over of the infected plant.





## SUMMARY

A species of *Pythium* with filamentous sporangia was isolated from rotting fruits of *Hibiscus esculentus* L. collected at Podanur, Coimbatore District, South India. A detailed study of the fungus showed it to be a form related to *Pythium indigofera*, *P. deliense* and *P. aphanidermatum* but possessing certain characteristics which mark it as different from these three species. On account of these differences, it is considered a new species and named *P. indicum*. Inoculation experiments showed it to have a wide host range. Only one other species of *Pythium*—*P. de Baryanum* Hesse—has been so far recorded on *Hibiscus esculentus*.

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# STUDIES ON THE MORPHOLOGY, PHYSIOLOGY AND PARASITISM OF THE GENUS *PIRICULARIA* IN MADRAS\*

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[Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., (Lond.) F.A.Sc.]

THE genus *Piricularia* belonging to the group *Hyphomycetes* is well known as the causal organism of the 'blast' disease of rice (*Oryza sativa* Linn.), 'ragi' (*Eleusine coracana* Gaertn.), and 'tenai' or the Italian millet (*Setaria italica* Beauv.) bringing about a heavy loss of food grains—as heavy as 76% or more (McRae, 1922). Other important hosts are the banana ('pitting disease'—Hoette, 1936; Van Hook, 1926), *Triticum vulgare* Vill. (Anstead, 1924), *Theobroma cacao* Linn. (Saccardo, 1875) and *Zingiber officinale* Rosc. (Nisikado, 1927). Several grasses also serve as hosts.

The genus has a wide distribution, being present in all tropical regions, especially the rice-growing tracts. Work on *Piricularia* has been carried out in all these regions, chiefly Italy, Japan and India and rice being the most important of the hosts, *Piricularia* on rice (called *Piricularia oryzae* Cav.) has received most attention. This form which causes the 'brusone' of rice (as the disease is called in Italy) was first recorded in Italy by Briosi e Cavara (1892) and much work has since been done on this fungus. In Japan work on 'blast' of rice (called 'Ine Imochibyo') was started by Hori (1898) and was continued by Kawakami (1901, 1902) and Miyake (1909, 1910). Much useful work on the host parasite relationship, leaf anatomy in relation to resistance, entry of the pathogen into the host, viability, breeding for resistance and methods of control was being done in research centres like Hokkaido and Formosa by numerous workers. Nisikado (1917, 1927) has made an attempt to assign a few Japanese isolates of *Piricularia* from different hosts to their systematic position within the genus with the help of their morphology, physiology and parasitism. In India the Madras Department of Agriculture has been tackling the 'blast' disease of cereals since 1918, when it was first recorded (McRae, 1920) as a serious outbreak in the Tanjore delta; much work has been done on the economic side of the problem, viz., devising methods of control, breeding for resistance, varietal trials for selecting resistant varieties of cereals and cultural practices to eliminate the disease (McRae, 1920–1923; Sundaraman, 1922–1936; and Thomas, 1930, 1931 and 1936–1941).

\* Formed part of a thesis accepted for the M.Sc. degree of the University of Madras.

The present communication includes a study of the morphology, physiology and parasitism of four isolates of *Piricularia* isolated from important cereals like *Oryza sativa* Linn., *Setaria italica* Beauv., and *Eleusine coracana* Gaertn., and the grass *Digitaria marginata* Link.

#### MORPHOLOGICAL STUDIES

*Materials and Methods.*—*Piricularia* was isolated from infected leaf tissues of rice (*Oryza sativa* Linn.—strain Adt. 10), 'ragi' (*Eleusine coracana* Gaertn.—strain E.C. 593 of the Millets Specialist, Coimbatore), 'tenai' or the Italian millet (*Setaria italica* Beauv.—local Coimbatore variety) and *Digitaria marginata* Link by single spore isolation method. Stock cultures were maintained on oat-meal-agar slants and on sterilised leaf bits of the respective hosts in Roux tubes. All work, which necessitated the study of fresh mycelia or spores direct from the hosts, was done during November to January, when the crop at Coimbatore showed maximum infection in the fields.

*Symptoms of infection.*—In rice, 'ragi', 'tenai' and *Digitaria* the fungus causes the characteristic leaf spots. In rice and 'ragi', in addition to the leaf spots in their younger stages, there is a darkening of nodes and necks of earheads after the flowering of the crops. The blackening of the necks and nodes is, however, absent in the case of 'tenai' and *Digitaria*. That the attack by *Piricularia* is restricted to the foliage in *Digitaria* sp. has been observed by Hansford (1943) in Uganda.

*Nature of leaf-spots.*—Spindle shaped dark-brown leaf spots are met with on leaves of rice, 'ragi' and *Digitaria* while spots on the leaves of 'tenai' are more or less circular. The spots invariably appear on either side of the mid-rib. On leaves of rice and 'ragi' the spots are found to be 1 to 3 cm. long. Weather conditions favouring development, neighbouring spots coalesce and very long spindles are formed and the leaf tissue rots in the middle and gets torn. The spots are greyish in the centre and brownish in the periphery. In 'tenai' the leaf spots are smaller and scattered. The spots are about 2 to 5 mm. in diameter, light brown in the centre and dark brown in the periphery.

*Mycelia.*—There is no appreciable difference between the mycelia of the different isolates. They are all thin, hyaline and straight when young. In older cultures they are thicker and attain a slightly brownish tinge and are variously contorted, developing swellings. In all the isolates the breadth of the mycelium varies from 1.4 to 5.8  $\mu$ .

*Conidiospores.*—The spores of all the isolates are hyaline and top-shaped. They are mostly three-celled (rarely two- or four-celled). The

spores may be straight or slightly bent. Some of them are very long and narrow while some are fairly broad. In any case the spores are longer than broad. They are borne in a scorpioid manner on conidiophores which are hyaline, their septa being very prominent. The fungus puts forth the conidiophores through the stomata of the affected parts of the plant and conidia are exposed to the air. In leaves the conidia are met with in the spotted region both on the upper side and on the lower side of the leaf-blade but more abundantly on the upper side. The spores vary in length from about 19 to 37  $\mu$  and in breadth from 7 to 15  $\mu$  and show no appreciable difference between the isolates.

The following table gives the average measurement of 200 spores taken of the four isolates:

TABLE I  
(Measurements expressed in microns)

<i>Piricularia</i> from		Spores from the living host		From sterile leaf bits	
		Length	Breadth	Length	Breadth
Rice ..	Mode	26.56	12.45	24.90	11.62
	Mean	29.22	12.63	26.35	11.58
	Range	20.75 to 37.35	10.79 to 14.94	21.58 to 32.37	9.13 to 14.11
' Tenai ' ..	Mode	24.90	12.45	24.90	11.62
	Mean	24.00	12.00	23.70	11.37
	Range	19.92 to 31.54	9.96 to 13.28	19.92 to 30.50	9.13 to 14.11
' Ragi ' ..	Mode	24.50	11.40	24.20	11.40
	Mean	23.60	11.20	23.40	11.30
	Range	19.50 to 30.00	9.50 to 14.50	19.30 to 30.20	9.50 to 14.50
<i>Digitaria</i>	Mode	24.00	11.20	24.20	11.30
	Mean	23.80	11.00	23.60	11.10
	Range	19.20 to 30.30	9.40 to 14.50	19.30 to 30.00	9.50 to 14.40

In all the four strains the end cells of the spores germinate readily when kept in a drop of water in a Van Tieghem cell.

*Chlamydospores*.—These are almost alike in all the isolates, round in shape, thick-walled, varying from 4 to 10  $\mu$  in diameter. These are brownish green in colour. Both terminal and intercalary chlamydospores are met with.

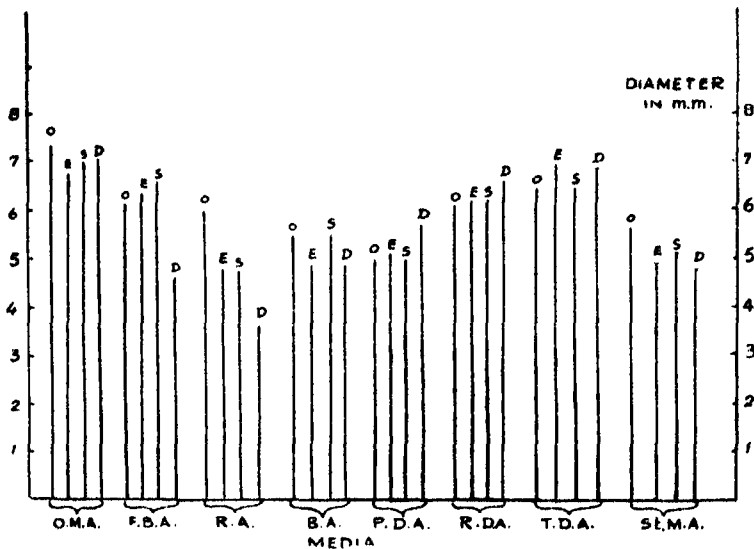
It is thus evident that morphological characters of the isolates do not afford much basis for their classification as they do not show significant differences. Nisikado (1917), though he has obtained similar results, has separated the strains on *Setaria italica* Beauv. into a new species—*Piricularia*

*Setaria* Nisikado, basing his classification on the size of the individual cells of the spores, the basal appendage and the size of the germinating hyphæ. But the variations obtained by him within the groups, viz., *Oryza* form and *Setaria* form, are too great and, therefore, any generalisation to include a particular spore in one group or the other may appear empirical and not rigidly scientific. Taking for instance the measurements of the conidia they vary from  $14$  to  $40\mu \times 6$  to  $13\mu$  in the *Oryza* form and  $14$  to  $35\mu \times 5$  to  $12\mu$  in the *Setaria* form. In the case of the individual cells of the conidium, taking the middle cell, it varies from  $4.8$  to  $12.0\mu$  in the *Oryza* form and  $4.8$  to  $8.3\mu$  in the *Setaria* form. It will now be difficult to say whether spores measuring say between  $14$  and  $35\mu$  in length or  $5$  and  $12\mu$  in breadth or having middle cells measuring between  $4.8$  to  $8.3\mu$  belong to the *Setaria* or the *Oryza* form. The importance of taking morphological evidence in collaboration with physiological needs, therefore, no emphasis and this will be discussed at a later stage.

#### PHYSIOLOGICAL STUDIES

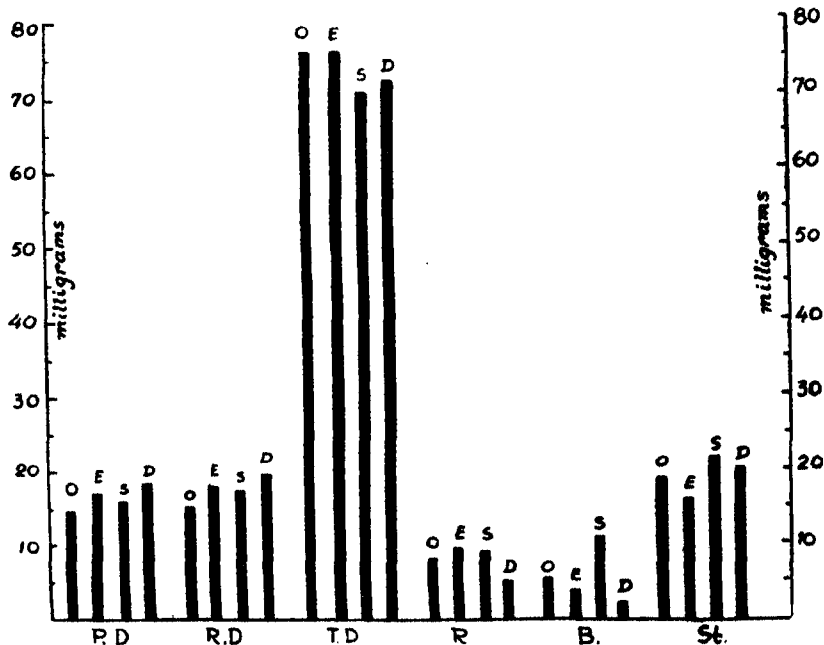
*Materials and Methods.*—The cultures of the four isolates used for the morphological studies were used for the physiological studies as well. Throughout the investigation standard mycological technique was followed and all the experiments were conducted at a constant temperature of  $30^{\circ}\text{C}$ . unless otherwise specified. For all the experiments a synthetic medium (Ramakrishnan, 1941) of the composition, glucose  $10\text{ gm.}$ , peptone  $6\text{ gm.}$ ,  $\text{K}_2\text{HPO}_4$   $1.75\text{ gm.}$ ,  $\text{MgSO}_4$   $0.75\text{ gm.}$ , water  $1\text{ litre}$ , agar  $2\%$  (for all solid media) was used unless otherwise stated. This is referred to as the Standard medium. In the case of growth studies care was taken to transfer equal quantities of the inoculum to the media in dishes or flasks. For obtaining the dry weight of mat the isolates were grown for a definite period in  $50\text{ ml.}$  of the medium in  $100\text{ ml.}$  flasks, the mat filtered through a gooch crucible, washed and dried in a hot water oven, to constant weight. All pH determinations were made with the help of a Quinhydrone Electrometric pH indicator and colour determinations with Ridgeway's (1912) colour nomenclature.

*Growth of the strains on media.*—Linear growth of the colonies of the four isolates on standard medium agar, Richard's agar, Brown's agar, oat-meal agar, French bean agar and the decoction agars made of the leaf material of rice, 'ragi' and 'tenai', was determined. The amount of mat produced by the isolates in the Standard medium, Richard's medium Brown's medium and decoctions of leaf material of rice, 'ragi' and 'tenai' was also determined. The results are represented diagrammatically below:



GRAPH I.—Showing the average daily growth in diameter of the colonies of the four isolates in millimetres on different agar media.

O.M.A. for oat meal agar; F.B.A. for French bean agar; R.A. for Richard's agar; B.A. for Brown's agar; P.D.A. for rice leaf decoction agar; R.D.A. for 'ragi' leaf decoction agar; T.D.A. for 'tenai' leaf decoction agar; St.M.A. for standard medium agar. O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.



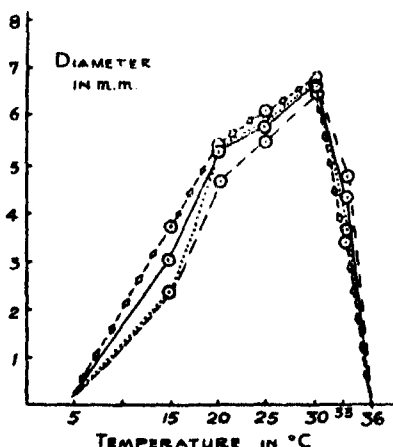
GRAPH II.—Showing the comparative amounts of mat produced by the isolates in different media.

(P.D., R.D., T.D., indicate leaf decoctions of rice, 'ragi' and 'tenai'. R., B., and St. indicate Richard's, Brown's, and Standard media.

O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.)

The results of the above experiments agree with those of Nisikado (1927) in that the Madras isolates also produce good growth on the decoctions of their host material as did the Japanese isolates. The Madras isolates produced yellowish olive colouration in synthetic media containing sugars. While linear growth is best on 'ragi'-leaf decoction agar, the isolates produce maximum amount by weight of mat in 'tenai'-leaf decoction agar, showing that measurement of linear growth in terms of increase in diameter of the colonies does not always give a correct idea of the amount of mat produced.

**Optimum temperature for growth.**—Linear growth of the colonies of the isolates and the weight of mat produced by them at different temperatures are given in the following graphs.



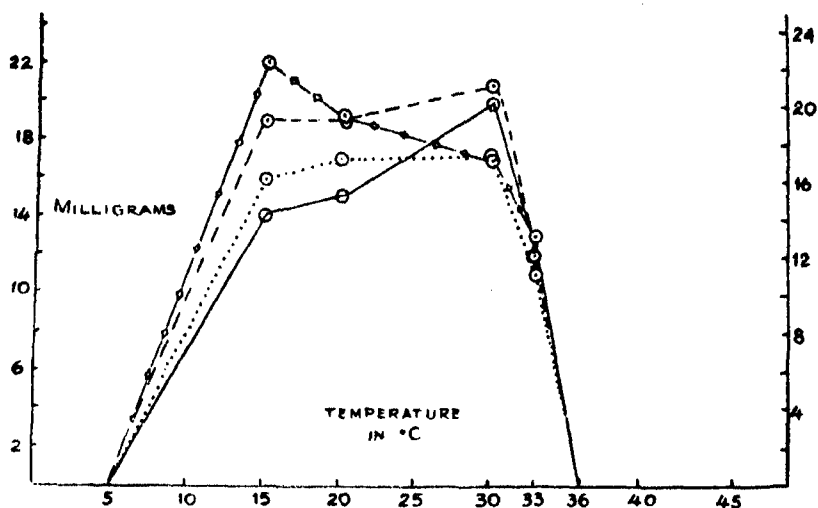
GRAPH III.—Showing the average growth in diameter of the colonies in millimetres at different temperatures.

————— isolate from *O. sativa*.  
 ..... isolate from *E. coracana*.  
 - - - - - isolate from *S. italica*.  
 -Δ-Δ-Δ-Δ isolate from *D. marginata*.

Thomas (1940) obtained best growth of a strain of *Piricularia*, isolated from 'ragi' at 29.5° C. Abe (1930) and Yoshii (1936) showed that 28° C. was the best for isolates from *O. sativa*. Nisikado (1927) has reported best growth of isolates from *Setaria* and ginger between 23 and 28° C., and the maximum temperature at which growth of the strains from rice was possible at 36 to 37° C.

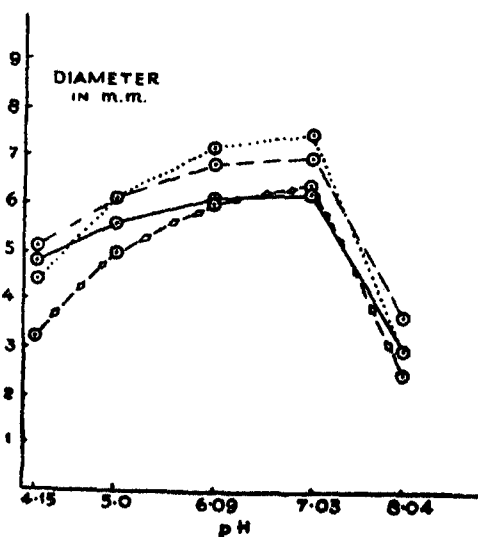
**Effect of pH of the medium on the growth of the isolates.**—The following graphs show the rate of linear spread of the isolates and the weight of mat produced by them in the Standard medium at different pH levels.





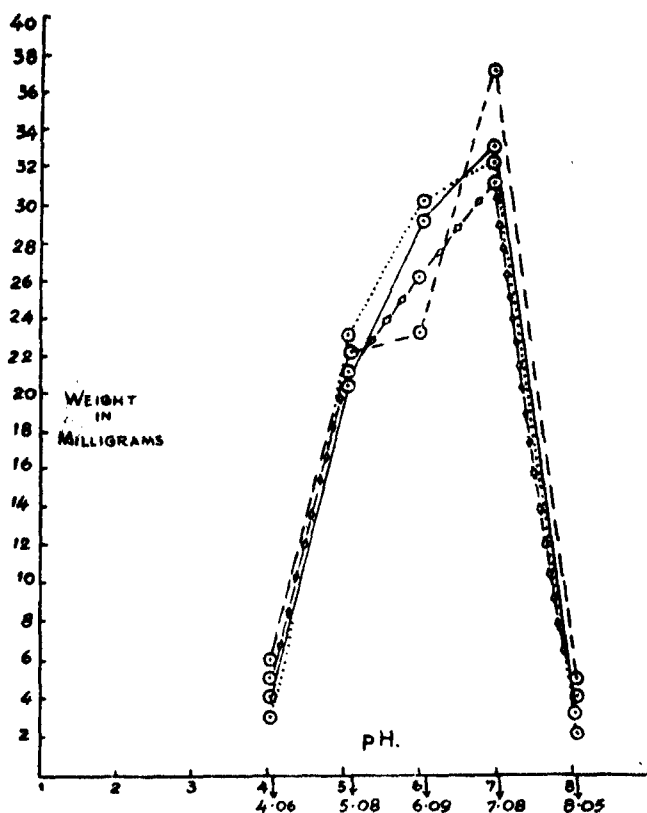
GRAPH IV.—Showing the comparative amounts of mat produced by the isolates in liquid medium at different temperatures.

— isolate from *O. sativa*.  
 ..... isolate from *E. coracana*.  
 - - - isolate from *S. italica*.  
 -△-△-△-△- isolate from *D. marginata*.



GRAPH V.—Showing the average rate of daily growth of colonies of the isolates at different pH levels.

— isolate from *O. sativa*.  
 ..... isolate from *E. coracana*.  
 - - - isolate from *S. italica*.  
 -△-△-△-△- isolate from *D. marginata*.



GRAPH VI.—Showing the comparative amounts of mat produced by the isolates at different pH levels.

— isolate from *O. sativa*.  
 ..... isolate from *E. coracana*.  
 - - - isolate from *S. italica*.  
 —△—△—△—△— isolate from *D. marginata*.

Nisikado (1927), with isolates from *O. sativa*, obtained best growth between pH values 5 and 10. A highly pathogenic strain grew best at pH 4.4. Thomas (1940) reports optimum growth of a strain from 'ragi' between pH 5 and 6. The present investigations show that round about pH 7 is the optimum for both dry weight and for radial spread irrespective of the isolates used. Though the variation in the weight of mat produced by the isolates is prominent the peak production of the mycelium both on dry weight basis and on radial spread was optimum for all the isolates at pH 7. During growth the isolates tend to bring the pH of the medium to round about 5 and 6.

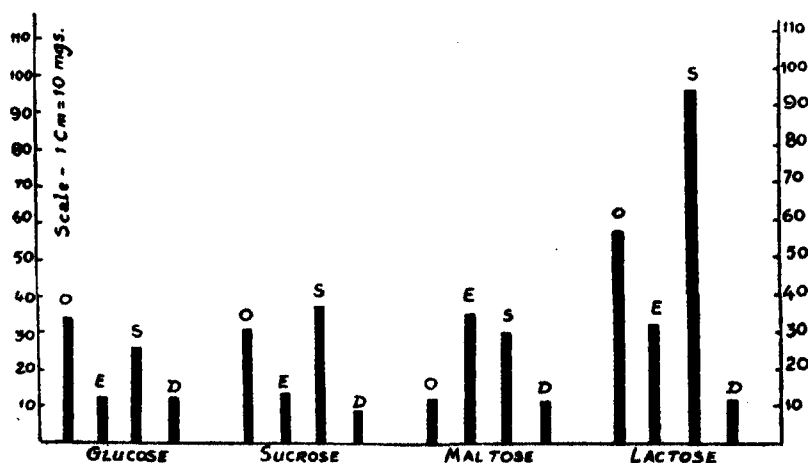
*Influence of different sources of carbon in the medium on growth.*—One of the following, viz., glucose, maltose, lactose, sucrose, soluble starch and

cellulose was tried as the source of carbon in the medium at the 2% level. The results are shown below:



GRAPH VII.—Showing the average growth in diameter of the colonies of the isolates in standard medium to which different carbohydrates were added.

O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.

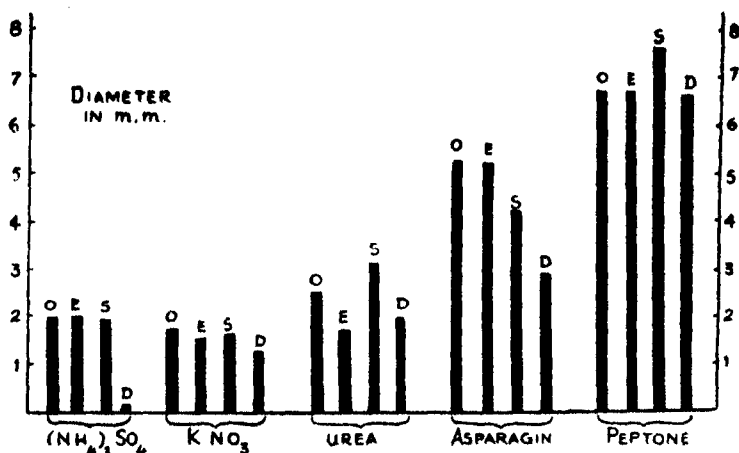


GRAPH VIII.—Showing the comparative amounts of mat produced by the isolates in media with different carbon sources.

O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.

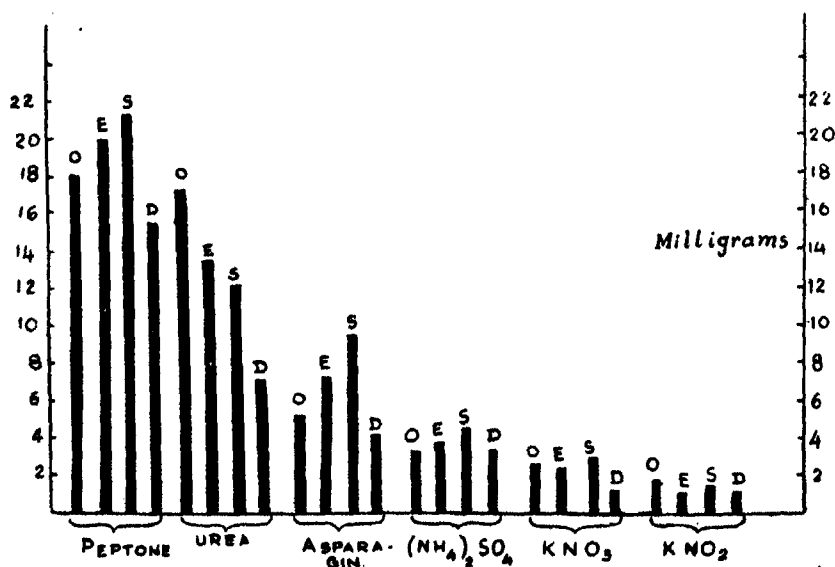
According to Tochinai and Nakano (1940) certain strains of *P. oryzae* appear to be capable of utilising higher alcohols like glycerine and mannite as the carbon source. Among the carbohydrates tried here they prefer maltose, soluble starch and glucose, in the order of their mention. Yoshii (1936) reports pectin as a good source of carbon for growth of *P. oryzae*.

**Influence of different sources of nitrogen on growth.**—The capacity of the isolates to utilise nitrogen from different sources was investigated using the following nitrogenous compounds in pure form: potassium nitrite, potassium nitrate, ammonium sulphate, urea, asparagin and peptone. Five grams of potassium nitrate was added to a litre of the standard medium.



GRAPH IX.—Showing the average daily increase in diameter of the colonies of the isolates in media with different nitrogen sources.

O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.



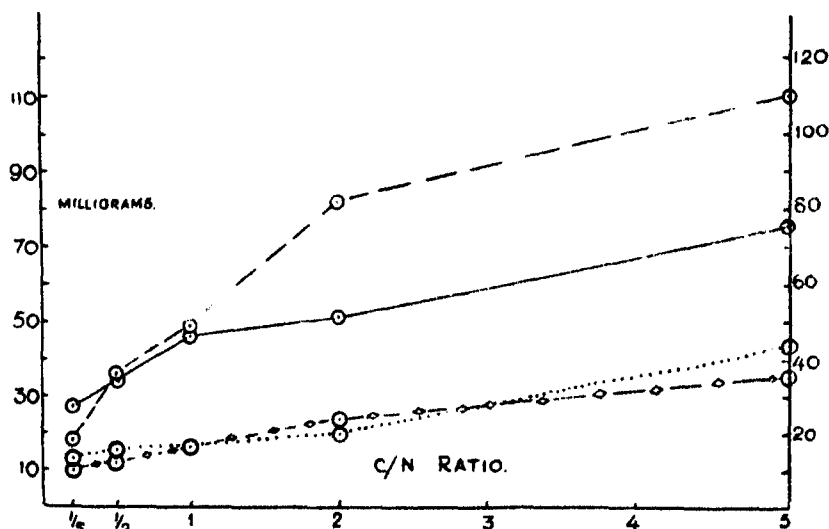
GRAPH X.—Showing the comparative amounts of mat produced by the isolates in media with different nitrogen sources.

O, E, S and D represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.

The other nitrogenous compounds were used in quantities calculated to contain an equivalent amount of nitrogen, *i.e.*, potassium nitrate 5 gm., potassium nitrate 4.25 gm., ammonium sulphate 3.1 gm., urea 1.5 gm., asparagin 3.3 gm., and peptone 5.9 gm. The above diagrams show the results.

Yoshii (1936) observed that *P. oryzae* did not reduce nitrate and was found to be injured by nitrite. Tochinal and Nakano (1940) observed that the best source of nitrogen for *P. oryzae* was peptone followed by sodium nitrate, asparagin, glutonic acid and acetamide. In the present investigation all the isolates do not favour potassium nitrite as the source of nitrogen in an agar substratum and growth is totally inhibited. Peptone gives the best growth both on solid and in liquid media showing that they prefer organic nitrogen to inorganic.

*Effect of different Carbon/Nitrogen ratios on the growth of the isolates.*— Different proportions of carbon and nitrogen sources were used keeping the total weight of glucose and peptone at 16 gm. per litre of the Standard medium. The experiment was conducted in liquid medium and the weights of mat produced by the isolates at different C/N ratios is given in the accompanying graph.



GRAPH XI.—Showing the comparative amounts of mat produced by the isolates in liquid medium at different C/N levels.

- isolate from *O. sativa*.
- ..... isolate from *E. coracana*.
- isolate from *S. italica*.
- Δ-Δ-Δ-Δ-Δ isolate from *D. marginata*.

It is found that the weight of mat produced increases as the C/N ratio increases and best growth is obtained at C/N = 5/1. Ramakrishnan (1941) also obtained similar results with isolates of *Colletotrichum falcatum*.

*Enzymes produced by the isolates during metabolism.*—The production by the isolates of the enzymes diastase, inulase, lipase, crepsin, amidase and trypsin was tested quantitatively by the method adopted by Uppal and Kulkarni (1937) in their work on *Fusarium*, based on the method of Crabill and Reed (1915). The production of maltase, sucrase, lactase and urease was tested *in vivo* by the method followed by Garren (1938) in his studies on *Polyporus abietinus*.

TABLE II

*Table showing the production of the different enzymes by the isolates*

Name of enzyme	Medium used	pH of medium	<i>Piricularia</i> isolated			
			<i>Oryza sativa</i>	<i>Eleusine coracana</i>	<i>Setaria italica</i>	<i>Digitaria marginata</i>
Diastase ..	Soluble starch	6.52	+	+	+	+
Inulase ..	Inulin	6.78	+	+	+	+
Lipase ..	Litmus cream agar	7.29	+	+	+	+
Erepsin ..	Casein agar	5.00	—	+	+	—
Amidase ..	Asparagin Rosalic acid agar	6.44	+	+	+	+
Trypsin ..	Egg albumen agar	6.35	+	+	+	+
Sucrase ..	Sucrose	6.52	+	+	+	+
Maltase ..	Maltose	5.93	+	+	+	+
Lactase ..	Lactose	6.26	+	+	+	+
Urease ..	Urea	8.80	—	—	—	—

+ shows production.

— shows non-production.

It is seen from these results that all the four isolates produce diastase, inulase, lipase, amidase, trypsin, surcase, maltase and lactase and do not produce urease. The isolates from *E. coracana* and *S. italica* produce erepsin, while those from rice and *D. marginata* do not. Yoshii (1936) has detected oxidase and dehydrase in cultures of *P. oryzae*.

#### CROSS INOCULATION STUDIES

Cultures of the isolates were maintained on sterilised leaf bits of the respective hosts. This facilitated a ready supply of spores whenever required as the fungi produced abundant conidia on these media and remained viable for long periods.

Seedlings were raised from healthy seeds in small pots. Garden soil was used for raising *S. italica* and *E. coracana* while soil from rice fields was used for raising rice and *Digitaria* seedlings. In each experiment 25 pots of

the seedlings were raised for each host inside glass cages which had been previously disinfected with a spray of 2 in 1,000 mercuric chloride solution, five pots in each cage. When the seedlings had grown to a height of about a foot, the seedlings were thinned out so that each pot contained about four healthy seedlings. The chambers were all kept in the shade in the pot-culture house. The experiments were conducted during the months November to January as infection by *Piricularia* was maximum in the fields during these months at Coimbatore.

Spore suspensions of the isolates were prepared in sterile atomisers. The inside of the cages was given a spray of clean water with a sprayer to raise the humidity, as high humidity favours infection by *Piricularia*. The spore suspensions of the isolates were sprayed on the plants in the four chambers each chamber receiving the spores of one of the isolates. The cages were kept closed. The plants in the fifth chamber formed the uninoculated controls.

Observations were made daily for the characteristic symptoms of infection by *Piricularia*. The symptoms, namely the leaf spots, appeared within 4 to 7 days after inoculation. The experiments were repeated thrice over two seasons. The following hosts of *Piricularia* were taken up:

1. *Oryza sativa* Linn. .. rice strain G.E.B. 24 of the Paddy Specialist, Coimbatore.
2.                   "                   ..                   Adt. 10.
3. *Eleusine coracana* Gaertn. 'ragi' strain E.C. 593 of the Millets Specialist, Coimbatore.
4. *Setaria italica* Beauv. .. 'tenai' local Coimbatore variety.
5. *Digitaria marginata* Link. .. seeds obtained from the Millets Specialist, Coimbatore.

Variety G.E.B. 24 of rice is a fairly resistant strain evolved by the Government Economic Botanist, Coimbatore. Adt. 10 is a very susceptible variety grown in the deltaic tracts of the Tanjore District of Madras.

The results are given below:

<i>Piricularia</i> isolated from	Hosts tried				
	Rice G.E.B. 24	Rice Adt. 10	<i>Eleusine coracana</i>	<i>Setaria italica</i>	<i>Digitaria marginata</i>
<i>O. sativa</i> ..	-	+	-	-	-
<i>E. coracana</i> ..	-	-	+	+	-
<i>S. italica</i> ..	-	-	+	+	-
<i>D. marginata</i> ..	-	+	+	-	+
Control ..	-	-	-	-	-

+ indicates infection.

- indicates non-infection.

It may be mentioned here that the isolate from *E. coracana* when infecting *S. italica* forms circular spots on leaves while the isolate from *S. italica* forms spindle-shaped leaf spots on *E. coracana* when infecting it. This shows that the nature of the leaf spot is due to certain qualities of the leaves of the host and not of the pathogen.

It may be concluded from the results of the experiments that the isolate from rice is distinct in that it infects only rice. The isolates from 'ragi' and 'tenai' each infects the host of the other in addition to its own. The isolate from *Digitaria*, in addition to its own host, infects 'ragi' and rice. These observations agree with those of Thomas (1940), who reports that the strain from 'ragi' and 'tenai' both infected 'ragi' and 'tenai' but not rice. Nisikado (1927) was not also able to get infection on grasses and cereals other than rice with *Piricularia* isolated from rice.

#### DISCUSSION AND CONCLUSION

There has been endless controversy and difference of opinion whether characters relating to morphology alone should form the basis for classification of the fungi into groups or whether their physiology, pathogenicity and host relationship should also be taken into consideration. Where there is marked morphological difference between the given members of the genus, it is an easy matter. But, when morphological differences are not very marked or are absent, the question must be asked whether classification should be based on such hair-splitting differences as variation of one or two microns in spore and mycelial measurements.

Turner (1940) compared *Ophiobolus* sp. from oats with isolates of *O. graminis* Sacc. from wheat, and found it difficult to distinguish one group from the other with the help of physiological characters. On the other hand marked differences were noticed in the length of the ascospores of the isolates from wheat and the ones from oats. All the isolates, however, produced similar symptoms in susceptible hosts. In spite of the differences in the morphological characters and in the host relationship, Turner (1940) considered the isolates from oats only as a new variety of *O. graminis* Sacc., viz., *O. graminis* Sacc. var. *Avena* Turner, because of their similarity in cultural behaviour and symptoms produced in the susceptible hosts.

Leonian (1932), working on the pathogenicity and viability of *Fusarium moniliforme* Sheldon, deplors the common notion that dissociants "played havoc with taxonomy while on the contrary they are of inestimable aid in bringing order out of chaos. We have been in the habit of describing the species according to its morphological characters; we have usually failed to make intensive cultural studies, and have endeavoured to formulate fundamental



truths by superficial observations. To many of us form and size of spores and reproductive bodies constitute the sum total of the mycological concept, and the vital life processes of the fungi have little taxonomic value in our scheme of classification."

Leonian (1925) in an earlier work employed certain physiological features as manifest on solid agars and the host relationship to overcome the uncertainty of a purely morphological classification of some *Phytophthoras*, for the specific distinction within the genus were very limited, as its members exhibited remarkable uniformity of morphological characters. He believes that the average of all the morphological, physiological and pathological features should form the specific sphere.

Padwick (1939), while criticising Wollenweber and Reinking (1935) for their "indiscriminate use" of morphological and physiological characters in their scheme of classification of the *Fusaria*, advocates the adherence to their system for, he says, "Inadequate though it may be, there is nothing better." He would base specific rank upon characters easily recognisable "under standard conditions available in all moderately equipped mycological laboratories". According to him, classification based on the use of host parasite relationship must be given a rank lower than the species, for it demands the use of a pure line of host and definite conditions such as temperature and moisture as has been shown by workers at Wisconsin, and soil conditions as shown by Mundkur (1936) working with the cotton wilt in India.

In the light of the above and the results obtained during these studies, it might be considered how the four isolates studied could be grouped. Of the four isolates, the one on *Oryza sativa* Linn. has been given a specific name *P. oryzae* Br. e Cav. ever since 1892. Nisikado (1917) working on isolates of *Piricularia* from *O. sativa*, *Setaria* spp., *Zingiber* spp. and some grasses reports that these forms "showed many differences among themselves morphologically and physiologically" and has, on this basis given the form of *Piricularia* on *Setaria* spp. the status of a species namely, *P. Setariae* Nisikado. A reference to the data put forth by him by way of morphological differences would show that emphasis could not be laid on measurements of the kind envisaged. The variations of individual measurements within the groups, namely, *Oryza* form and *Setaria* form, are too great and, therefore, generalisation to include any particular spore in one group or the other, may appear empirical and not rigidly scientific. It does not, therefore, appear proper that the separation of the *Setaria* form should have been based on such data. The isolates from *Eleusine*

*coracana* Gaertn. and *Digitaria marginata* Link. have not been assigned their systematic status so far.

From the results of the present observations it may be said that a study of the morphological characters does not help in distinguishing between the four isolates, there being no characteristic difference in the mycelia, conidio-phores, conidia and chlamydospores. The only differences observed are in the nature of infection—(only foliar infection in the case of *Digitaria* and *Setaria*; and foliar, nodal and earhead infection in *O. sativa* and *E. coracana*, and the nature of the leaf spot, more or less roundish in *S. italica* and spindle-shaped in others). Even the nature of the leaf spots depends only on the host, as the *Setaria* isolate, when infecting *E. coracana* forms spindle-shaped spots and the *Eleusine* isolates when infecting *S. italica* form roundish spots. From the results of the physiological studies, the *Digitaria* isolate may be said to be distinct from the other three.

(1) It forms slate grey growth on oat-agar, while the others give pale gull grey growth. Unlike the other isolates, it does not grow well on Brown's agar.

(2) The *Digitaria* isolate does not very much favour cellulose as the carbon source, while the other isolates grow well in agar medium with cellulose as the carbon source.

(3) The *Digitaria* isolate does not grow in agar media with ammonium sulphate as the nitrogen source, while the other isolates, though they do not very much favour ammonium sulphate, grow fairly well with ammonium sulphate in the agar medium.

(4) 30° C. is the optimum temperature for growth, in liquid cultures of the isolates except the one from *Digitaria* which shows optimum growth at 15° C.

(5) Isolates from *E. coracana* and *S. italica* produce the enzyme erepsin, whereas the isolates from *O. sativa* and *D. marginata* do not.

It may thus be seen that the *Digitaria* isolate has certain physiological characters of its own.

It is very difficult to distinguish between the isolates from *O. sativa*, *S. italica* and *E. coracana* from their physiology, as they do not differ very much in their behaviour in culture media. The only differences are:

(1) The isolate from *O. sativa* differs from the other two by its inability to produce erepsin.

(2) The isolate from *E. coracana* shows better growth on agar medium with ammonium sulphate as the nitrogen source than with urea, whereas the others prefer urea to ammonium sulphate.

(3) In liquid medium maltose is the best source of carbon for the isolate from *E. coracana* while the other isolates grow best with lactose.

Cross inoculation experiments show that the *Piricularia* isolate from *O. sativa* is distinct from the other three isolates from *E. coracana*, *S. italica* and *D. marginata* in that it does not infect any host other than *O. sativa*. Nisikado (1927) also did not get infection with isolates from *O. sativa* on any host other than *O. sativa*.

The isolate from *S. italica* infects its host and *E. coracana* but not *D. marginata* or *O. sativa*. Similarly, the isolate from *E. coracana* infects in addition to its host *S. italica* and not *O. sativa* and *D. marginata*.

Nisikado (1917, 1927) did not study isolates from *E. coracana* and *D. marginata* nor did he use these hosts in his studies. He has doubted the ability of the isolates from *Setaria* spp. to infect *O. sativa*.

The isolate from *D. marginata* is able to infect *O. sativa* and *E. coracana* in addition to its host. It should be noted here that isolates from *O. sativa* and *E. coracana* do not infect *D. marginata*.

Summing up, the various *Piricularia* isolates can be classified into three groups:

(a) Isolates that attack only their natural hosts, e.g., isolate from *O. sativa*.

(b) Isolates that attack their own hosts and one other, e.g., isolates from *E. coracana* and *S. italica*.

(c) Isolates that attack their own hosts and two more, e.g., isolate from *D. marginata*.

If morphology could alone be depended upon as the basis of classification it will be impossible to place the isolates systematically. If Leonian's (1925) suggestion of taking the sum-total of all the characters, morphological, physiological and pathological, is to be followed, it may be necessary to study more forms before arriving at some conclusion. The system of Wollenweber and Reinking (1935) of studying physiological characters in addition to morphological is somewhat similar to Leonian's (1925) suggestion and, indeed, has been emphasised by Padwick (1939) who has recommended the use of characters easily recognisable under standard conditions in all moderately equipped mycological laboratories and this presumably includes cultural studies as well. Padwick (1939) has also admitted of a classification based on the host relationship, though as inferior in rank to the species, if a pure line of the host and definite conditions of temperature and moisture

were assured. In the present studies, the hosts used were the susceptible varieties and the experiments were conducted under conditions most favourable for infection (under ideal weather conditions in Coimbatore during the months November to January, when the crops showed maximum infection in the field) thus incorporating the suggestions of all the workers in the experimental technique.

Nevertheless, it has been found difficult, if not impossible to draw bold lines of demarcation between subtle differences that exist among the different isolates of *Piricularia* studied here. Although very prominent and specific differences between the physiological behaviour and degrees of pathogenicity have been noticed among the isolates, it has been found impossible to detect significant morphological differences. This study, without making extravagant claims, has established the importance of attacking problems of classification of fungal parasites from three angles, namely, physiology, pathology and morphology, and the final analysis of the results to be undertaken by studying the three aspects interdependently and not independently. Further work with larger number of isolates from more hosts that the fungus *Piricularia* is known to attack, possibly grasses under the monocots and other hosts like *Theobroma cacao* Linn. among the dicots would enable significant comparisons easier and may result in establishing different species or varieties of the same species. Until such time, this work enables us to conclude that the various strains of *Piricularia* studied here can only be considered from a broad evolutionary point of view and they have to be provisionally placed as physiological races within the genus and species *Piricularia oryzae*.

#### SUMMARY

The morphology of four isolates of *Piricularia* from *Oryza sativa* Linn., *Eleusine coracana* Gaertn., *Setaria italica* Beauv., and *Digitaria marginata* Link., has been studied. No appreciable difference in the morphological characters of the isolates was noticed.

The physiology of the isolates was studied. A study of their growth on different media, the optimum temperature and pH range for their growth was made. The effect of different sources of carbon and nitrogen and their different ratios in the medium on the growth of the isolates was investigated. The enzymes produced by the isolates were qualitatively tested for.

Cross-inoculation studies were conducted under optimum conditions for infection of the hosts.

The studies reported in this paper have established the importance of attacking problems of classification of fungal parasites from three angles,

namely, morphology, physiology and pathology and these aspects considered interdependently and not independently. With the results obtained an attempt has been made to classify the isolates within the genus.

#### ACKNOWLEDGMENTS

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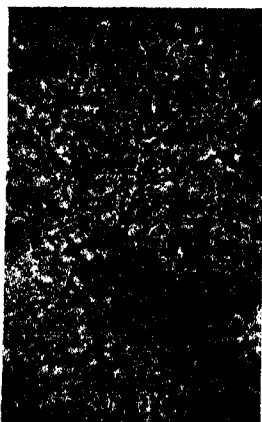
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## EXPLANATION OF PLATE

1. Photograph of two varieties of rice — one resistant and the other susceptible — seen side by side in an experimental plot at Coimbatore.
  - A. Susceptible variety completely destroyed by *Piricularia* attack.
  - B. Resistant variety unaffected.
2. Photomicrograph of mycelia from young cultures of *Piricularia* (mycelia straight and thin)  $\times 360$ .
3. Photomicrograph of mycelia from older culture (mycelia variously contorted)  $\times 360$ .
4. Photomicrograph of 3-celled top-shaped spores of *Piricularia oryzae* Br. e Cav.  $\times 230$ .
5. Rice (*Oryza sativa* Linn.) affected by *Piricularia* (spindle-shaped leaf spots and darkened neck, node and grains) — diagrammatic.
6. 'Ragi' (*Eleusine coracena* Gaertn.) affected by *Piricularia*. Note spindle shaped leaf spots with neighbouring spots coalescing. Also showing affected ear-heads — diagrammatic.
7. 'Tenai' (*Setaria italica* Beauv.) affected by *Piricularia* (Circular leaf spots, ear-heads not affected) — diagrammatic.

Note.—For photograph 1 the author is indebted to the Govt. Mycologist, Coimbatore and to Dr. T. S. Sadasivan.





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# THE ANATOMY OF THE VERTEBRAL COLUMN IN SERPENTES\*

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## I. INTRODUCTION

The vertebral column of Snakes and its associated parts deserve an intensive investigation on account of the many adaptations they show in consequence of the peculiar type of locomotion in this order. As functional

\* Part of a thesis approved for the Bh.D. Degree in Agra University.

limbs are absent, the backbone, ribs, the costal and cutaneous musculature and the ventral shields have had to take up the function of locomotion. This has resulted in a considerable number of interesting modifications.

The ophidian vertebral column is characterized by a great many peculiarities. The number of vertebræ is very large, the maximum so far recorded for living<sup>1</sup> species being 435 (Rochebrune, 1881). The division into well-marked regions is less distinct than in other vertebrates. Usually, all the pre-caudal vertebræ except the atlas and axis<sup>2</sup> bear ribs, and the caudal vertebræ have long 'transverse processes'. The successive vertebræ articulate not only by the pre- and post-zygapophyses and by the procœlous centra, but there are additional articulations present, the zygosphenes and zygantra. The neuro-central suture is absent and the hypapophyses may or may not be present. There are no chevron bones, but "the transverse processes of the caudal vertebræ have strong descending processes which have the same relation to the caudal vessels" (Sedgwick, 1905).

Although the general structure and development of the ophidian vertebræ have been the subject of many valuable contributions, little attempt has yet been made either to study them minutely in consecutive serial sections, or to study their precise relations to each other and to the various associated organs. The present contribution, therefore, aims to supplement our knowledge of the vertebral column in these respects and to lay down a foundation for further accurate work on it.

## II. HISTORICAL RÉSUMÉ

Although no previous investigator has studied the minute anatomy of the adult vertebra and the precise articulation of one vertebra with another in the Serpentes in consecutive serial sections, the general anatomy of the ophidian vertebræ has been known for a very long time. As far back as 1833, Jourdain described the main features of the vertebral column in *Coluber scaber*, while three years later (1836) D'Alton gave an account of the vertebræ of *Python*. Owen (1853) gave a fairly detailed account of the vertebræ of several snakes—*Python tigris*, *Boa constrictor*, *Crotalus horridus*, *Naia tripudians*, *Dirodon scaber*, etc., and a number of text-book writers (Meckel, 1821–33; Dumeril and Bibron, 1834–54; Carus, 1835; Cuvier,

<sup>1</sup> In an extinct species, *Archæophis proavus*, the vertebræ reach the enormous number, 565.

<sup>2</sup> Sedgwick (1905, p. 356) says, "all the pre-caudal vertebræ, except the atlas, carry ribs." This is not strictly accurate as the ribs are absent on the second vertebra also in Cobra and other snakes (Owen, 1866, p. 55).

1835; Schlegel, 1837; Grant, 1841; Straus-Durkheim, 1842-1855; Gegenbaur, 1862; Owen, 1866; Huxley, 1871; Sedgwick, 1905; Wiedersheim and Parker, 1907; Reynolds, 1913; Williston, 1925; Kingsley, 1925-26, Goodrich, 1930; Remane, 1936, etc.) have dealt with the subject. Peters (1872) described the vertebræ of the genus *Streptophorus*. Rochebrune (1881) studied the structure of the vertebræ and the characters of the various regions of the vertebral column in snakes, and observed the differences in the vertebræ of the various families, the number of vertebræ and their distribution in regions, and the role of the hypapophyses. Salle in the same year (1881) described the peculiar forked processes on each side of the vertebræ in the sacral region of snakes and discovered the lymph-hearts situated between them. Boulenger (1890, revised by Smith, 1943) made a number of stray observations on the occurrence of the hypapophyses on the vertebræ of numerous colubrid genera. Goette (1897) noted the composition of the reptilian vertebræ, including those of snakes. Kathariner (1898) reported on the structure of the elongated hypapophyses penetrating the œsophagus in *Dasypeltis* and interpreted their mode of action. Lubosch (1908, 1910 and 1913) scrutinised the joints between the vertebræ and their development. Wall (1921) found that the dorsal vertebræ of *Lycodon carinatus* (Boulenger in *Fauna*, 1890, p. 296) differed from the other species of *Lycodon*, in so far as their prezygapophyses were extended to form strong lateral expansions and their neural spines were expanded and divided into two by a longitudinal groove. He, therefore, separated this species generically from *Lycodon*. Emeljanoff (1925), Seemann (1926), Lubosch (1926) and Mosauer studied the peculiarities of the relation of the ribs to the vertebral column. Mookerjee and Das (1933) reported on the presence of a series of sub-central foramina in *Typhlops braminus*. Mahendra (1936) discovered that the odontoid is separate from the second vertebra and lies between the two lateral pieces of the atlas in *Typhlops braminus* and that the zygosphenes-zygantral arrangement in this snake is more primitive than in others. Dunn (1941) studied the skeleton of the blind snake *Anomalepis* and found that the vertebræ "in general closely resemble those of *Typhlops braminus* as figured by Mahendra," but that the odontoid is not a separate bone but a part of the axis.

### III. MATERIAL AND TECHNIQUE

All the material for the present study was collected in the suburbs of Agra, except that the specimens of *Enhydrina schistosa* were presented to Professor Beni Charan Mahendra by Dr. B. Sundar Raj, Director of the Government Fisheries, Madras. The following families were represented:

(a) *Typhlopidae*.—

- (1) *Typhlops braminus* (Daud.)—20 specimens.
- (2) *Typhlops porrectus*, Stoliczka—12 specimens.

(b) *Boidae*.—

Sub-family: *Pythoninae*

*Python molurus*, (Linn.)—2 specimens.

Sub-family: *Boinae*

- (1) *Eryx johani johani* (Russell)—12 specimens.
- (2) *Eryx conicus* (Schneid.)—6 specimens.

(c) *Colubridae*.—

Series—AGLYPHA

Sub-family: *Colubrinae*

- (1) *Lycodon aulicus* (Linn.)—15 specimens.
- (2) *Ptyas mucosus* (Linn.)—3 specimens.
- (3) *Coluber diadema* Schleg.—6 specimens.
- (4) *Natrix piscator* (Schneid.)—2 specimens.

Series: PROTEROGLYPHA

Sub-family: *Elapinae*

- (1) *Bungarus caeruleus* (Schn.)—6 specimens.
- (2) *Naja naja* (Linn.)—6 specimens.

(d) *Hydrophiidae*.—

*Enhydrina schistosa* (Daudin)—2 specimens.

The vertebral column was studied by three methods:

(a) *Preparation of whole skeletons*

In formalin-preserved specimens, muscles could be removed readily without further preparation, the only precaution taken being to moisten them now and then with water and to keep them wrapped in a wet cloth while not working. Freshly killed specimens, however, were either macerated with caustic potash, or prepared without maceration. In the former case they were skinned and fixed in 90 per cent. alcohol for two or three days, before treating with potassium hydroxide. In the latter, the superfluous muscles were removed after skinning, and the individuals preserved in 90 per cent. alcohol for two or three days after which the muscles were removed. It was found helpful to immerse the specimens in hot water while removing the muscles and to whiten the skeletons by hydrogen peroxide.

(b) *Alizarin Preparations:*

The method used is the one already described by Mahendra (1936, p. 129).

(c) *Transverse and Longitudinal Sections:*

The procedure for preparing sections varied according to the material under preparation. Young specimens were fixed either in Bouin's solution or in Carnoy's fluid, and decalcified in Ebner's fluid. Larger ones, both fresh and formalin preserved, were decalcified with the following fluid, recommended by Mukerji (1937) for insects.

Saturated solution of picric acid in 90 per cent. alcohol	..	75 parts
Formalin	.. .. .	25 parts
Strong Nitric Acid	.. .. .	5 parts

The fluid acted both as fixative and decalcifying agent and several better than nitrated alcohol. The average time required for the decalcification of a piece from the middle body of an adult snake varied from three to four days. The fluid was changed once or twice each day so as to accelerate its action and to ensure complete decalcification. After washing in 70 per cent. alcohol the specimens were dehydrated in 95 per cent. and absolute alcohols, cleared in xylol and embedded in paraffin. Embedding generally required forty-eight to seventy-two hours in case of medium-sized individuals, and only six to seven hours in small specimens like *Typhlops*.

In certain cases it was found that the blocks had become too hard for sectioning and required softening by the exposure of a surface by trimming and by immersion overnight in a glycerine-alcohol mixture (90 c.c. of 60 per cent. alcohol mixed with 10 c.c. of glycerin).

Sections were stained with Ehrlich's Hæmatoxylin and alcoholic Eosin, Borax carmine and Picro-indigo Carmine, and Mallory's triple stain.

Diagrams were made with an Abbe's Camera Lucida, using an eyepiece no. 2 or 3 and an objective no.  $\frac{2}{3}$  or  $\frac{1}{3}$  (Swift).

#### IV. DIVISION OF THE VERTEBRAL COLUMN INTO REGIONS

Meckel (1821), Carus (1835), Dalton (1836), P. Grant (1841), Straus-Durkheim (1842-1855), Siehold and Stannius (1849), Owen (1866), Hoffmann (1890), Sedgwick (1905), Reynolds (1913), Williston (1925) and others distinguish only two regions in the ophidian vertebral column: a *precaudal* region called by some the presacral region (Hoffmann, 1890), and a *caudal* region called also the postsacral (Hoffmann, 1890). As the sacrum is absent

in all snakes except the *Leptotyphlopida*, the terms sacral and presacral are not tenable.

Rochebrune (1881), however, distinguished five regions: the cervical, the thoracic, the pelvic, the sacral and the coccygeal regions. He based this division on the longer or shorter form of the centrum, on the inclination of the upper arch, the location of the rib-processes, the shape of the transverse processes, the inclination of the hypapophyses, etc. While Rochebrune's regions are certainly distinguishable from each other, the differences are rather slight and very variable. In no case can they be regarded as equivalent to the regions of the vertebral column in other vertebrates. Rochebrune's classification was, therefore, not adopted by later workers.

Although the division of the ophidian vertebral column into two regions (*precaudal and caudal*) is worth retaining, we can distinguish several sub-regions in each of these regions as follows:—

#### A. *The precaudal region*

(a) *The cervical subregion* composed of only the first two vertebræ, *atlas* and *axis*. Not only are these peculiar in their form and structure, but as shown by Rathke's researches (1839) they also develop in a manner different from the other vertebræ.

(b) *The thoracic subregion*, consisting of all the vertebræ that follow the *axis* and bear *hypapophyses*.

(c) *The lumbar subregion*, consisting of the vertebræ situated between the thoracic and the anterior subcaudal regions and generally devoid of hypapophyses. Even when hypapophyses are developed on the lumbar vertebræ, we can distinguish this sub-region from the thoracic by a number of minute differences in the form and structure of the vertebræ (Rochebrune, 1881), particularly by the fact that the hypapophyses in the thoracic vertebræ are long and stout structures, while in the lumbar ones they are reduced to a ridge, rarely exceeding the base of the condyle.

#### B. *The caudal region.*

In all the species studied by me, the caudal region can be divided into the antero-caudal, mid-caudal and postero-caudal sub-regions. As a detailed account of these sub-regions has already been published by me (1941), there is no need of repeating it here.

### V. GENERAL STRUCTURE OF THE VERTEBRA

In order to give a general idea of the structure of an ophidian vertebra, a trunk vertebra of the common sand-snake, *Eryx johni johni* (Russell) is

described here in detail. This is followed by an account of the salient features in which the other vertebræ in this snake differ from those of the trunk region.

(a) *Component parts.*

A typical vertebra of the trunk region in *Eryx johni johni* (Russell) as in other Tetrapod vertebrates is composed of a ventral piece, the *centrum*, a dorsal hoop-shaped structure, the *neural arch*, and a number of processes, the *apophyses*.<sup>3</sup> The line of demarcation (*the neurocentral suture*) between the centrum and the neural arch is completely lost in the adult. Each vertebra is distinctly broader than high and thus corresponds to the dorso-ventrally compressed appearance of the body in this snake.

The centrum is not round but rather compressed dorsoventrally: its anterior face bears a concavity or socket (the *glenoid* or *cotyloid* cavity), its posterior one a convexity (*condyle*), the vertebræ being procœlous. Minute observation shows that neither the concavity faces directly forwards nor the convexity directly backwards. The concavity looks "a little downward, from the greater prominence of the upper border: the well-turned prominent ball terminates the back part of the centrum rather more obliquely, its aspect being backward and upward." (Owen, 1866, p. 53).

The *neural arch* is formed by a pair of lateral bones, the *neurapophyses*, which vary considerably in their size and appearance as well as in the form, height and direction of the median process, the *processus spinosus* (neural spine), developed on them.

(b) *Processes*

The processes developed on the ophidian vertebra are of three kinds: (i) those which serve to articulate the consecutive vertebræ with each other; (ii) those which provide attachment to the ribs, and (iii) those which serve for the insertion of muscles.

(i) *The processes for the articulation of successive vertebræ* in snakes are the *prezygapophyses*, *postzygapophyses*, *zygosphenes* and *zygantra*.

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<sup>3</sup> Owen distinguishes two kinds of parts in a vertebra: (1) *autogenous*, i.e., those which develop from independent centres of ossification and (2) *exogenous*, i.e., those which grow from previously ossified parts. The autogenous parts of a vertebra are its 'elements', the exogenous parts are its 'processes'. The vertebral elements are the centrum, the neurapophyses, the neural spine, the pleurapophyses, the hæmapophyses and the hæmal spine; while the exogenous parts are the diapophyses, the parapophyses, the zygapophyses, the anapophyses, the metapophyses, the hypapophyses and the epapophyses. (P. 27, 28).



Regarding the pre- and post-zygapophyses in snakes, Owen (1866) observes that "the base of the neural arch swells outward from its confluence with the centrum, and develops (*sic*) from each angle a transversely-elongated zygapophysis; that from the anterior angle.... looking upward; that from the posterior angle,.... downward." While this description is applicable to the prezygapophyses, the postzygapophyses are developed rather above the point at which the base of the neural arch joins the centrum.

A feature not noted by previous authors is the presence of a lateral projection on the *prezygapophyses* of each side, pointing outwards and slightly forwards and serving for the attachment of certain muscles. This projection seems to correspond to the *metapophysis* (*Processus mammillaris*) of mammals. It is present in all the species of snakes examined by me.

The *zygosphene* is a wedge-shaped process developed from the anterior part of the base of the neural spine and bearing two smooth flat facets on its ventro-lateral surfaces. It is received "into a cavity the 'zygantrum' excavated in the posterior expansion of the neural arch, and having two smooth articular surfaces to which the zygosphenal surfaces are adapted" (Owen, 1866, p. 55).

(ii) *The processes for the attachment of ribs* were called by Owen (1866) 'diapophyses' if they were developed on the neural arch, and 'parapophyses' if they were developed on the hæmal arch. This terminology was accepted by Williston (1925), who said, "a longer or shorter process on the sides of the arch for the support in part or wholly of the ribs is known as a *diapophysis*. A like process or facet on the side of the centrum anteriorly for articulation of the head of the rib is called a *parapophysis*. Either is commonly called a transverse process, and the same term is often applied to a like process on the sides of the caudal vertebræ, of which probably the anterior ones, at least, in all cases are merely co-ossified ribs."

Remane (1936) while accepting the term '*parapophysis*' for the process articulating with the capitulum of a dichocephalic<sup>4</sup> rib and '*diapophysis*' for that articulating with its tuberculum, coins another term '*synapophysis*' to designate the process articulating with the head of a syncephalic rib. The synapophysis is formed by the fusion of a parapophysis with a diapophysis.

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<sup>4</sup> The following terminology for ribs, given by Remane (1936, p. 152), has been accepted here :

Dichocephalic ribs (Williston) = two-headed, with capitulum and tuberculum.

Capitulocephalic ribs = one-headed, head = capitulum.

Tuberculocephalic ribs = one-headed, head = tuberculum.

Syncephalic ribs = one-headed, head = capitulum + tuberculum.

(iii) *The processes for the insertion of muscles* are the *processus spinosus*, the *metapophyses* and the *hypapophyses*.

The *processus spinosus* commonly called the *neural spine* is a median unpaired projection pointing upwards from the place where two neurapophyses meet each other. It is present throughout the vertebral column in *Eryx johni* and is rather short and truncated. It is obliquely pointed backwards in the first few vertebræ and directed almost vertically in others.

The *processus spinosi* is absent in the *Typhlopidae*. In other families we can distinguish two types of this process: *spine-like* and *ridge-like*. The former are long or short, bluntly-pointed, truncated spines, connected loosely enough to permit a fair latitude of separation from each other by the flexion of the vertebral column. The latter, preferably called 'neural ridges,' are flat plate-like crests, extending vertically from the anterior faces of the vertebræ almost to their posterior ends and connected to each other by rigid ligaments.

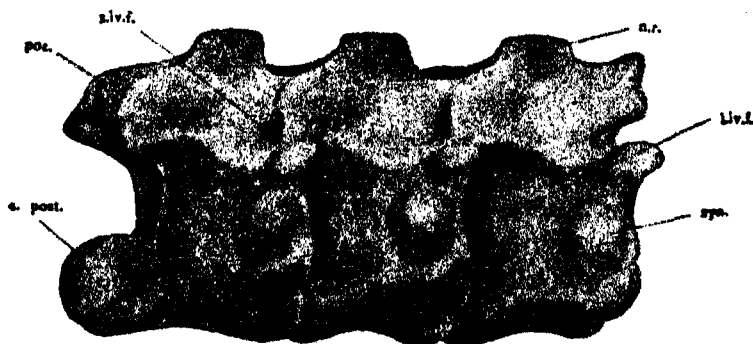
The *metapophyses* have already been described in connection with the prezygapophyses (*vide supra*, page     ).

The *hypapophyses* are short median processes developed on the mid-ventral aspect of the centrum and pointed obliquely backwards. They are rudimentary or absent in the middle trunk region in *Eryx johni*, but are fairly well developed in the anterior quarter of the vertebral column.

### (c) *Foramina*

There are typically two sets of apertures in the vertebral column: the *intervertebral* and the *intravertebral*.

The inter-vertebral set (Text-Fig. 1) unlike that of other vertebrates, when seen from outside, appears to be composed of two apertures on each



TEXT-FIG. 1. Lateral view of the trunk vertebræ of *Eryx johni johni* (Russell) ( $\times 3$ ).  
*c. post.*, posterior face of the centrum; *l.w.f.*, inferior inter-vertebral foramen; *n.r.*, neural ridge; *poz.*, post-zygapophysis; *s.w.f.*, superior inter-vertebral foramen; *syn.*, synapophysis.

side—a dorso-lateral one, which may be called the *superior inter-vertebral foramen* and a lateral one which may be called the *inferior inter-vertebral*. The superior inter-vertebral is bounded dorsally by the articulation of the zygosphenes with the zygantrum and ventrally by that between the pre- and the post-zygapophyses. The inferior inter-vertebral is situated on each side ventrad to the zygapophysial articulation between the bases of the neurapophyses of any two contiguous vertebrae. These foramina, when viewed from inside the vertebra, are seen to coalesce into one foramen, opening into the neural space.

The *intra-vertebral foramina* are a pair of minute apertures situated ventrally in the middle of each centrum on either side of the longitudinal line. They are not present throughout the vertebral column.

## VI. MINUTE ANATOMY OF THE TRUNK VERTEBRA

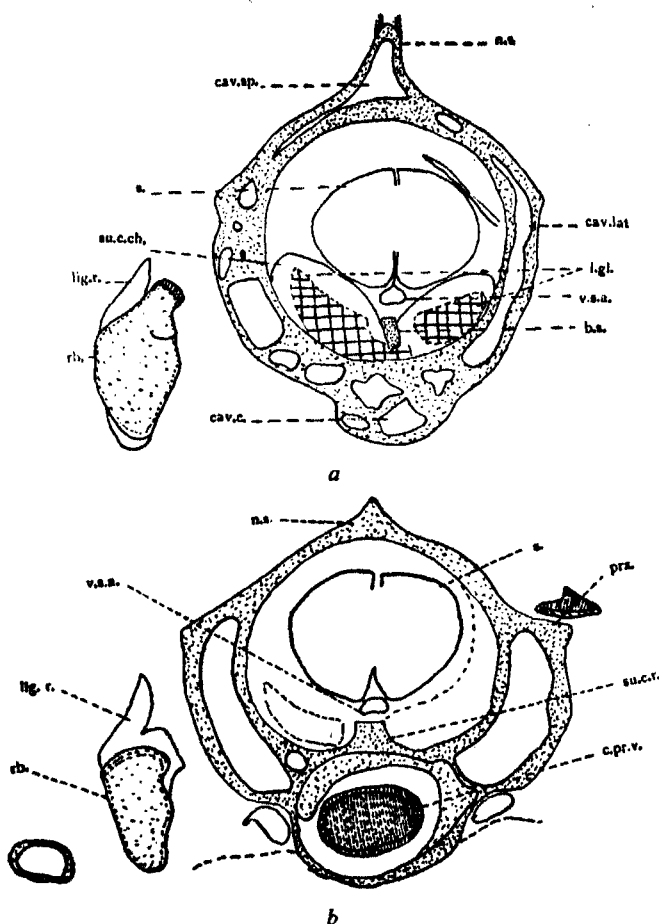
### (a) *Eryx johnei johnei* (Russell)

A minute study of serial transverse sections of the trunk vertebra of *Eryx johnei* not only confirms the general structure already described, but reveals a number of new features. As no previous investigation has studied the vertebra of an adult snake minutely in series of consecutive sections, it is advisable to record my observations in detail. Amongst the new features discovered, one may particularly mention the precise relation of a vertebra to the contiguous ones, the internal anatomy of the various parts, the nature of the articulations, and the presence of a supra-central ridge and of two supra-central cavities.

Each vertebra may be divided into three regions, situated one behind the other: First, the *anterior region* articulating with the posterior region of the preceding vertebra; secondly, the *middle region* devoid of articulating processes; and thirdly, the *posterior region* articulating with the anterior region of the succeeding vertebra.

If we begin with a transverse section passing through the middle region of a vertebra (Text-Fig. 2, A), we find that the vertebra forms almost a round ring with a bluntly pointed projection, the *neural spine* or *processus spinosus*, in the middle of its dorsal surface, and a dilatation of its ventral wall, the *centrum*. Inside the wall are a number of irregular spaces, filled up with bone marrow and communicating with each other. These perhaps serve to lighten the weight of the vertebra. Although the marrow-spaces run into each other when traced forwards or backwards, they may be divided roughly into three kinds: (a) spinous, (b) lateral, and (c) ventral. The *spinous space* is present only in the middle region of the vertebra; it gradually

tapers and finally disappears, when traced forwards or backwards. It runs at places into the lateral marrow-spaces. The lateral and ventral spaces, unlike the spinous, extend from the anterior region of the vertebra to its posterior, and become fairly enlarged at certain places by coalescence.



TEXT-FIG. 2. Consecutive transverse sections through the middle region of a trunk vertebra of *Eryx Johni* ( $\times 35$ ). Section B is anterior to A.

*b.s.*, supra-central bony septum; *cav.c.*, marrow space in the centrum; *cav. lat.*, lateral marrow space; *cav.sp.*, spinous marrow space; *c.pr.v.*, centrum of the preceding vertebra; *l.gl.*, lymphoid gland; *lig.r.*, ligament of the rib; *n.s.*, neural spine; *prz.*, prezygapophysis; *rb.*, rib; *s.*, outline of the spinal cord; *su.c.ch.*, supracentral chamber; *su.c.r.*, supra-central ridge; *v.s.a.*, ventral spinal artery.

Besides the marrow-spaces, the middle region of the vertebra shows the presence of a short bony septum which is developed immediately above the dorsal surface of the centrum and separates two ventro-lateral spaces inside

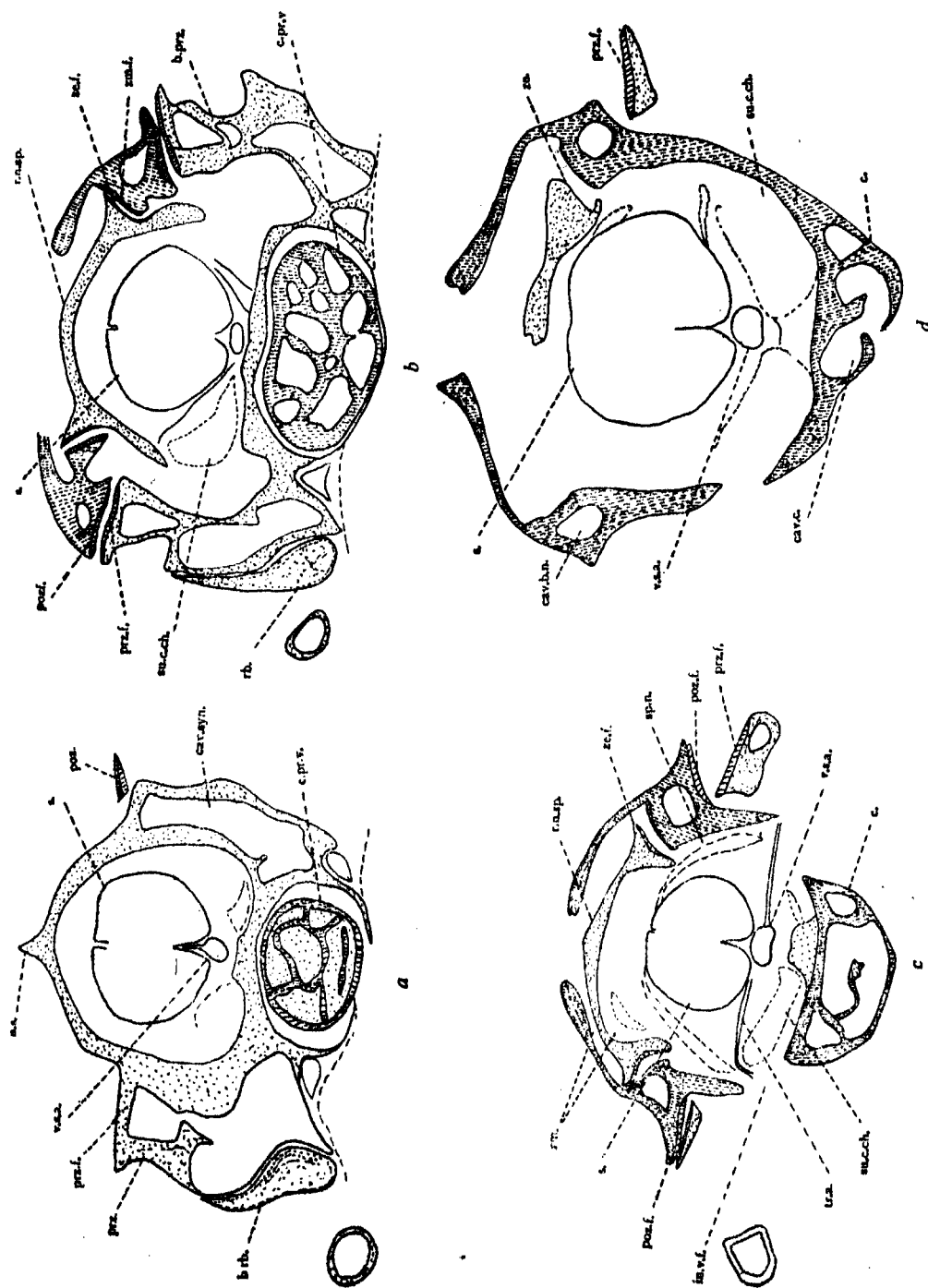
the neural cavity underneath the spinal cord. This septum which has not been noted by previous authors may be called the *supra-central ridge* and the spaces, the *supra-central chambers*. The latter are bounded dorsally by the dura mater, laterally by the bases of the neurapophyses, mesially by the supra-central septum, and ventrally by the centrum. They contain a lymphoid gland, not hitherto described.

A little anterior to the plane of the transverse section described above, the basal portions of the neurapophyses are much dilated, each bearing a large lateral space (Text-Fig. 2, *B*). The rounded tip (*condyle*) of the centrum of the preceding vertebra is visible inside a concavity (*glenoid cavity*) in the centrum of the present vertebra. The neural spine is much shorter and devoid of a marrow space.

The next section (Text-Fig. 3, *A*) shows the base of the synapophysis articulating with the base of a rib. The articulating surface of the rib is not rounded, but shows a dorsal concavity united with a ventral convexity. The synapophysis, in order to accommodate such a surface, bears a rounded prominence in its upper half and a deep excavation in its lower. Above the synapophysis there is a horizontal flat articular surface, the *pre-zygapophysial facet*. The condyle of the preceding vertebra which lies inside the glenoid cavity of the present one, shows several lines of ossification in its inside, the portions between the lines of ossification being cartilaginous. The supra-central ridge is remarkably shortened in height and shows a wide base.

Further forwards, in the section (Text-Fig. 3, *B*) in which we can make out the post-zygapophyses of the preceding vertebra, the neural spine is altogether absent and the roof of the neural cavity is formed by a dome-like structure with its sides projecting outwards and upwards into horn-like particular processes, not described so far. Each of these processes, which may be called the *zygosphenal processes*, bears a facet (*zygosphenal facet*) on its ventro-lateral aspect, articulating with a similar but oppositely directed facet on the post-zygapophysial region of the preceding vertebra. The latter may be called the *zygantral facets*. This articulation has not been noted by previous authors, but an examination of serial sections as well as dry preparations leaves no doubt about its presence; the soft parts of the joint are very well defined in preparations stained with Mallory's triple stain.

In addition to the *zygosphenal* and *zygantral* facets, a number of other peculiarities can be made out in this section. A gap between the pre-zygapophysis and the neural arch on each side shows that the connecting portion between these does not extend quite up to the anterior end of the vertebra.



TEXT-FIG. 3. Transverse sections through the trunk vertebra of *Eryx johni*, showing the articulating region. Section D is the anteriormost ( $\times 35$ ) at the base of the pre-zygapophysis; b.r.b., base of the rib; c., centrum; cav.syn., marrow space inside the synapophysis; cav.b.n., cavity at the base of the neuropophysis; in.v.f., intervertebral foramen; poz., post-zygapophysis; poz.f., post-zygapophysial facet; prz.f., pre-zygapophysial facet; r.n.sp., dome-like roof of the neural space without the neural spine; sp.n., spinal nerve; tr.a., transverse infra-spinal teryar ze.f., zygosphenal facet; zm., zygantrum; zm.f., zygantral facet. Other abbreviations as in previous figures.

Part of the neural arch of the preceding vertebra domes over the present one, thus partially enclosing a space, the *zygantrum*, in which the neural arch of the latter forms the *zygosphene*. The post-zygapophysis of the preceding vertebra is seen to bear a horizontal plate with its lower surface abutting against the upper surface of the pre-zygapophysis. The centrum of the preceding vertebra, is about twice as broad as high, has a slightly concave upper surface, and bears a number of prominent lines of ossification internally. The glenoid facet accommodating this centrum has its upper edge stoutly-formed, while the lower border is thin and delicate.

Still anteriorly (Text-Fig. 3, *C* and *D*), the present vertebra disappears gradually while the preceding vertebra assumes the typical ring-shaped appearance. The first parts to disappear are the ventral and lateral regions while the *zygosphene* and the prezygapophyses are visible in sections even farther ahead. The upper border of the cup-shaped centrum projects a little further forwards than the lower (Text-fig. 3, *B*). A conspicuous gap on each side between the centrum of the preceding vertebra and its neurapophysis indicates the position of the intervertebral foramen.

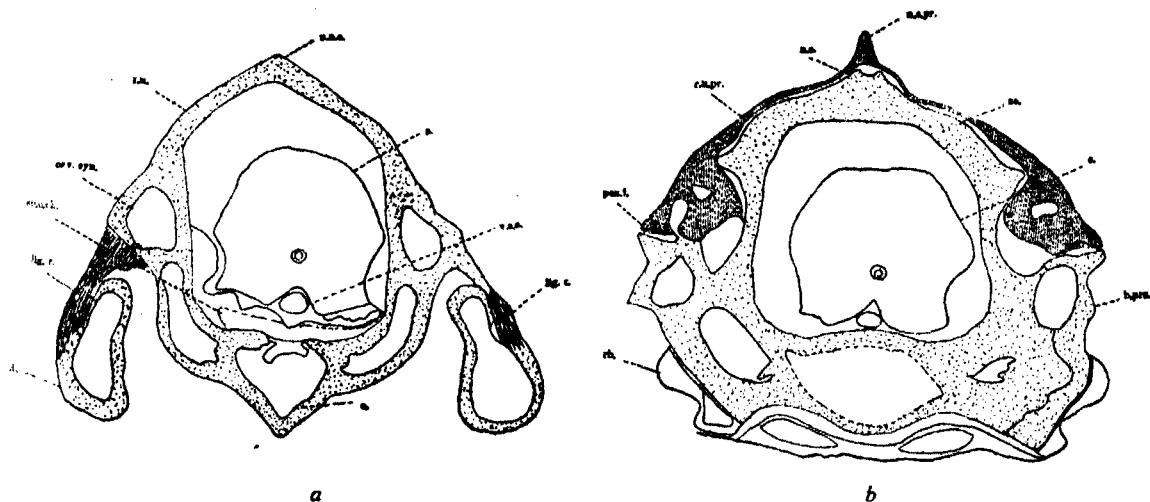
(b) *Ptyas mucosus* (Linn.)

The trunk vertebræ of *Ptyas mucosus* are not dorso-ventrally compressed like those of *Eryx johani*, but in conformity with the general configuration of the body, are distinctly taller than broad. The processus spinosus is developed along the whole antero-posterior length and may, therefore, be more accurately called the neural ridge. The spinous marrow space is confluent with the lateral marrow-spaces, which pass on at certain places into the marrow space of the centrum.

The rib articulates with the base of the neurapophysis on either side of the centrum and a distinct tendon (Text-Fig. 4, *A*) is seen to extend from the outer and upper border of the rib to a rounded projection on the side wall of the vertebra. At this level, the section shows no marrow space in the region of the neural spine or the arching part of the neurapophysis, but the part of the neurapophysis giving attachment to the rib is more strongly developed and bears two spaces separated from each other by a horizontal partition. The sections passing immediately posterior to the middle region of the vertebra shows a condition reverse to this relation. In them the neural spine and the upper parts of the neurapophyses are more strongly developed and bear marrow spaces, while the basal parts of the neurapophyses are relatively less stout and are devoid of marrow.

The intervertebral articulations by the zygapophysial and zygosphenal joints are similar to those in *Eryx johani*, but it is important to note that the

posterior border of the preceding vertebra (Text-Fig. 4, B) forms a complete arch over the anterior border of the vertebra under description, so that the zygosphenes have a completely wedge-shaped appearance. The zygosphenal and zygantral articular facets show the same relations as in *Eryx johni*.



TEXT-FIG. 4. Transverse sections through a trunk vertebra of *Ptyas mucosus* anterior to the plane figured in Text-Fig. 8. Section *B* is anterior to *A* ( $\times 45$ ).

*b.n.s.*, base of the neural spine; *n.s.pr.*, neural spine of the preceding vertebra; *r.n.*, roof of the neural space; *r.g.pr.*, roof of the neural cavity of the preceding vertebra. Other abbreviations as in previous figures.

(C) *Lycodon aulicus* (Linn.)

The vertebræ of this snake on the whole are similar to those of *Ptyas mucosus* in their general form and structure. The neural ridge, however, has no marrow-space inside, but bears on its ventral aspect a wedge-shaped cavity, forming an extension of the neural space into the spine and lodging a part of the supra-neural glands, described by me elsewhere. Such a cavity, as far as I know, is not present in other snakes.

The vertebral wall is more solidly built than in other species and bears only the following spaces: (a) a pair of rounded lateral marrow-spaces at the bases of the neurapophyses; (b) the marrow-space in the centrum; and (c) the synapophysial marrow-space. In the middle region of the vertebra the synapophysial spaces coalesce with the marrow-space in the centrum to form a long channel extending from one side of the vertebra to another. Such a feature is also present in *Typhlops braminus* (*vide infra*, page     ).



On the ventral wall of the centrum there is a minute projection, the *hypapophysis*. It differs from that in other snakes in having a mid-ventral split at certain places.

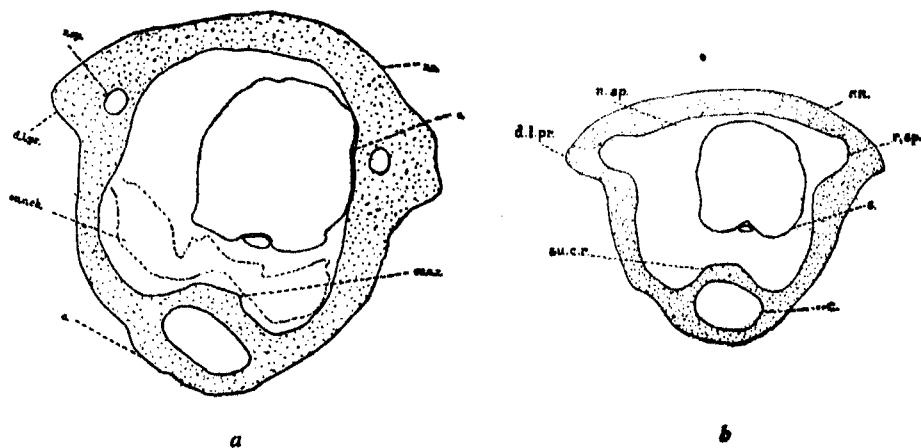
The attachment of the ribs to the vertebra and the articulations between successive vertebræ are essentially like those of *Eryx johni*.

(d) *Typhlops braminus* (Daud.)

The trunk vertebræ of *Typhlops braminus* show the following important differences from those of *Eryx johni*:

(1) The vertebræ in their middle region are as broad as tall, but in the other regions they are almost twice as broad as tall, appearing consequently to be dorso-ventrally flattened.

(2) The neural arch along the whole vertebra forms an evenly curved dome over the neural space, the *processus spinosus* being absent. The section passing through the middle of a vertebra (Text-Fig. 5, A) shows a projection dorso-laterally on each side and a rounded space in the neuropophysial wall at this height. The projections are actually the extensions of the pre-zygapophyses posterior to their articular surfaces, and each of the rounded spaces, when traced backwards (Text-Fig. 5, B) opens into the neural space by a wide aperture.



TEXT-FIG. 5. Consecutive transverse sections through a trunk vertebra of *Typhlops braminus* ( $\times 175$ ). Section D is the anteriormost.

*d.l.pr.*, dorso-lateral projection on the neural arch; *r.n.*, roof of the neural space; *r.sp.*, rounded space in the neuropophysial wall. Other abbreviations as in previous figures.

(3) The sub-central foramen occurs as a wide cleft on the ventral surface of the centrum in the middle line. This has already been described by Mookerjee and Das (1933) and Mahendra (1935).

(4) The supra-central ridge, although remarkably broad, is very slightly elevated (Text-Fig. 5, A).

(5) The projecting centrum of the preceding vertebra shows no lines of ossification enclosing cartilaginous portions, but is a compact structure.

(6) There are no marrow-spaces in the wall of the vertebra, except for a large space inside each synapophysis. This may be called the *synapophysial marrow-space*. In one of the vertebræ sectioned, the synapophysial space of each side was in communication with a narrow canal, extending into the centrum and opening into the glenoid facet. The significance of this canal is not clear, although it appears to be an abnormality. Such a canal has not been previously recorded.

(7) The articular surface of each synapophysis, unlike that in *Eryx johni* is merely rounded, abutting against a cup-shaped facet on the proximal end of the rib.

(8) The disposition of the zygapophyses, zygosphenal processes and zygantral facets is almost similar to those in *Eryx johni*.

(9) Mahendra (1936) noted that "the zygantrum is not a cavity or even depression at all, as in other ophidians, but a minute articular surface; and the zygosphen, which is merely a slight conical process, just distinguished at either end of the more or less flattened arch between the prezygapophyses, overlaps (rather than fits into) the zygantrum. The zygosphenes and zygantrum cannot be said to be dorsal to the zygapophyses, but are at a level with them."

This description, based on an examination of alizarin-stained whole preparations of the vertebræ, may be modified and supplemented on the basis of serial transverse sections as follows:

The zygantral space is not enclosed dorso-mesially as there is a wide notch on the posterior face of the neural arch in each vertebra. The *zygantral facets*, however, are well defined; they appear in the form of a pair of dorso-lateral pockets on the posterior part of the neurapophyses at level with the post-zygapophyses. The *zygosphen*, consequently, does not appear to fit into a cavity as in other snakes, but forms a pair of lateral articular processes which lie under flap-like projections on the post-zygapophysial region of the preceding vertebra.

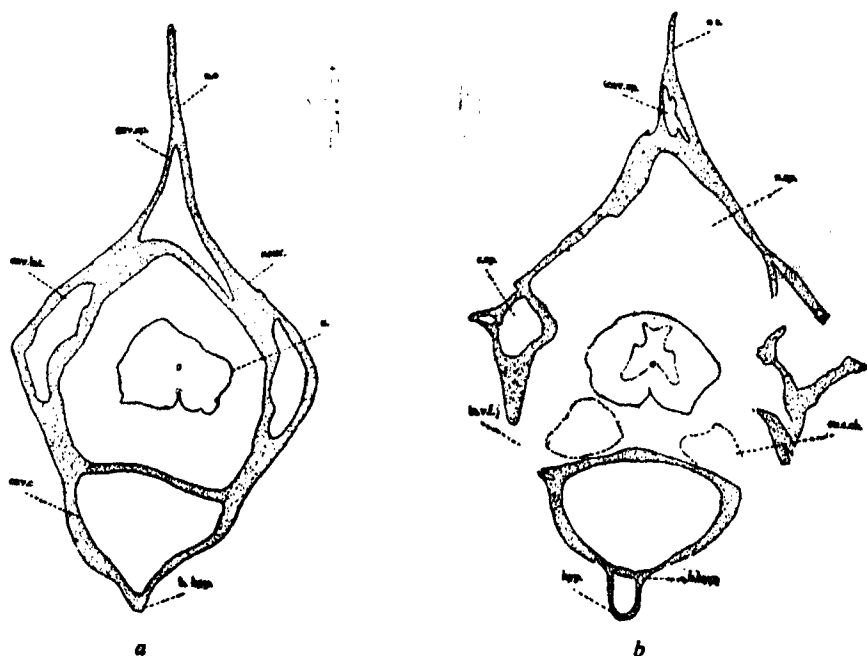
(e) *Enhydrina schistosa* (Daud.)

The vertebræ of the sea-snake *Enhydrina schistosa* differ considerably from those of other snakes. Even in the trunk region they are conspicu-

ously elongated in the dorso-ventral axis and rather compressed laterally. They are nearly twice as high as broad, a great part of this height being due to the greatly elongated neural spine.

In sections passing approximately through the middle of a vertebra (Text-Fig. 6, *A*), the neural spine is seen in its entire height. It contains a large triangular marrow-space at its base and possesses a solid rod-like distal part. The wall of the vertebra on each side contains a lateral marrow-space, which may or may not be connected to the spinous space by a channel.

The centrum in this region shows a small ovoid projection at the mid-ventral line (Text-Fig. 6, *B*) which represents the hypapophysis. Its marrow cavity is not separated from that of the centrum all along, although an ossified bar is present at the level where the hypapophysis is distinctly seen. The dorsoventrally compressed centrum bears no supra-central ridge as in other snakes.



TEXT-FIG. 6. Transverse sections through the middle of a trunk vertebra of *Enhydrina schistosa* ( $\times 35$ ).

*b.hyp.*, base of the ovoid projection representing the hypapophysis; *hyp.*, hypapophysis; *neur.*, neurapophysis; *n.sp.*, neural space. Other abbreviations as in previous figures.

The sections showing articulation between two successive vertebræ present a number of differences from those of other species. In the mid-dorsal region the point of the neural spine of the preceding vertebra projects

over the articulating parts of the vertebræ, which means that the neural spine extends considerably backwards from each vertebra. The neurapophyses bear a rounded space on each side corresponding to that in *Typhlops*. The zygantral facet is connected to the neural arch above by a weak and slender piece, which often breaks in sections.

The articulation of the rib lies not lateral but rather ventro-lateral to the centrum, and the synapophysial articular surface, consequently, faces somewhat obliquely downwards. The ribs, consequently, arch more distinctly downwards on each side of the vertebral column to enclose the laterally compressed body.

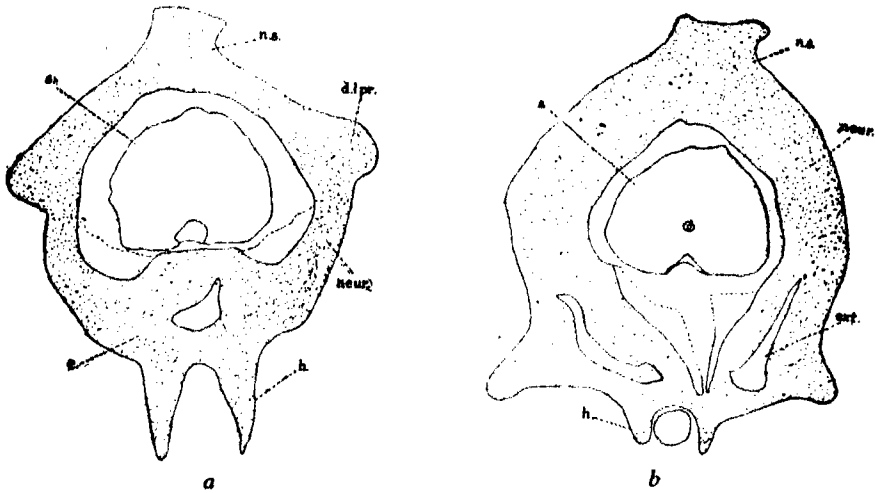
In several sections a split in ossification exists in the neurapophysis on each side just where its vertical basal portion passes on to form the arching neural roof. The split is seen to be no artifact, but a denite structure even under high powers.

#### VII. MINUTE ANATOMY OF THE CAUDAL VERTEBRA

As the caudal vertebræ of *Eryx johni* differ so considerably from those of other snakes that they cannot be regarded as typical of the tail in the Serpentes, I have thought it best to select for the present study two colubrid species (*Lycodon aulicus* and *Natrix piscator*) and one sea-snake (*Enhydrina schistosa*). In all these snakes the caudal vertebræ, although constructed on the same general plan as the trunk vertebræ, differ from them in their general configuration, the presence of a pair of hæmapophyses, the absence of the rib articulations and a simplification of the zygosphenal and zygapophysial articulations.

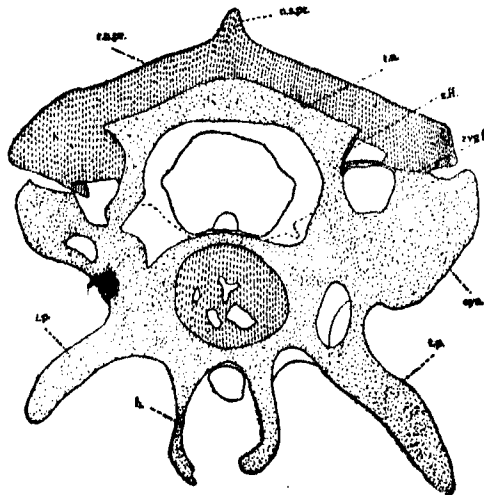
##### (a) *Lycodon aulicus* (Linn.)

In the middle region of the vertebra (Text-Fig. 7, A), the centrum bears a pair of conical processes (*hæmapophyses*) projecting downwards. The neurapophyses and the processus spinosus are stoutly built, being devoid of marrow-spaces. There is a rounded lateral projection on each side of the neurapophysis midway between its base and summit. A little anterior to this plane the section (Text-Fig. 7, B) shows a pair of short ventro-lateral processes (*the transverse processes*) in addition to the ventrally directed hæmapophyses. Still anteriorly (Text-Fig. 8) we find the intervertebral articulations of the centrum, and the zygapophyses, showing almost the typical disposition. But curiously enough, the neural roofs of the two articulating vertebræ are in close contact throughout, so that the zygosphenæ and zygantra are not in the form of articulations.



TEXT-FIG. 7. Transverse sections through a posterior caudal vertebra of *Lycodon aulicus* ( $\times 130$ ). Section *B* is anterior to *A*.

*ext.*, extension of the synapophysial cavity; *h.*, haemapophysis. Other abbreviations as in previous figures.



TEXT-FIG. 8. Transverse section passing through two successive posterior caudal vertebrae of *Lycodon aulicus* at the plane of intervertebral articulation ( $\times 55$ ).

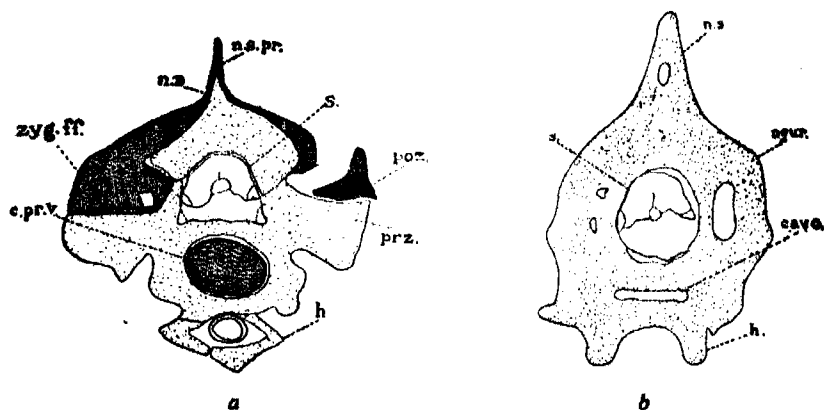
*t.p.*, transverse process; *z.f.*, zygosphenal and zygantral facets closely applied; *z.p.f.*, zygapophysial facets closely applied. Other abbreviations as in previous figures.

While the foregoing structure is visible in the great majority of the caudal vertebrae, a few vertebrae in the neighbourhood of the anus possess peculiar biforked processes on each side, enclosing the lymph hearts. Salle (1881), called these processes *lymphapophyses* and classified them into costal

or costotransversal, according as the fork occurs on the ribs or on the *Processus costotransversarii*. They are not constant in number and may differ on the two sides of an individual. In *Natrix natrix*, the number varies from two to seven, the most frequent number being three to five. In the costal lymphapophyses both the limbs lie on the capitulum, sometimes attached to a short neck-piece developed on it; while in the costo-transversal lymphapophyses, the limbs are developed on the base of a processus transversus. The lymphapophyses enclose ventrally a space which tapers towards the anterior and posterior ends and is deepest and broadest in the middle. The limbs serve for the attachment of certain muscles.

(b) *Natrix piscator* (Schn.)

The postero-caudal region of the tail of *Natrix piscator* has essentially the same structure as that of *Lycodon quilius*. At places, however, the hæmapophyses meet together in the mid-ventral line. In the mid-caudal region, the hæmapophyses have each a peculiar hook-shaped appearance (Text-Fig. 9, A) at the plane of intervertebral articulation, but they (Text-Fig. 9, B) form a nodule-like process on the ventral aspect of the centrum at the middle plane of the vertebra. The transverse processes in this region are much elongated, extending outwards and downwards from the ventro-lateral borders of the centrum. They are bifurcated at their basal end to form extremely short dorsal and ventral limbs. The dorsal limb is attached to the base of the neurapophysis and the ventral one to the side of the centrum. The limbs are separated by a clear suture from the body of the vertebra to which they are attached.



TEXT-FIG. 9. Transverse sections of the mid-caudal vertebrae of *Natrix piscator* ( $\times 40$ ). Abbreviations as in previous figures.

(c) *Enhydrina schistosa* (Daud.)

The caudal vertebræ of the sea-snake, supporting as they do the laterally undulating paddle-shaped tail, are amongst the most peculiar vertebræ in the Serpentes. They are elongated in the dorso-ventral axis. Their neural spines are extremely high and pointed, supporting the dorsal fin-like outgrowth of the tail. Their hæmapophyses project downwards to a great distance below the centrum and are stout structures, protecting the contents of the hæmal space. The centra are strongly built. The neurapophyses are devoid of marrow-spaces. The antero-posterior length of the articular region of the vertebra is much shortened as compared to its middle region.

If we begin our study with the pre-caudal region, we find the vertebra approaching that of the trunk in its general configuration and structure, but it is almost as broad as high, has a pair of slender hæmapophyses not meeting mid-ventrally and is covered over at its anterior margin by the backwardly projecting neural arch of the preceding vertebra.

The vertebræ of the mid-caudal region differ from those of the pre-caudal in three important respects. Firstly, they have a tall pointed neural spine which is almost as high as the rest of the vertebra and bears a large spinous marrow-space inside. Secondly, the hæmapophyses are a pair of rod-shaped structures projecting downwards and outwards from the ventral aspect of the more or less triangular centrum and are widely separated from each other at their distal ends. Thirdly, a conspicuous blunt transverse process extends outwards and downwards from each side of the centrum in the middle region of the vertebra.

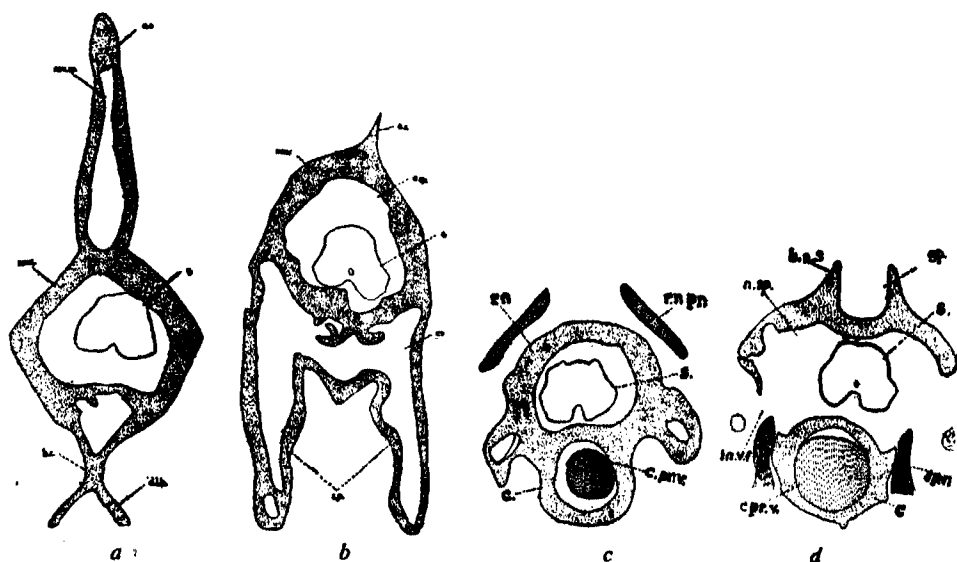
Each vertebra of the postero-caudal region shows a considerable difference in its configuration as we trace it in transverse sections from behind forwards. The posteriormost part of the vertebra anterior to its articulating region (Text-Fig. 10, A) is characterized by a lofty processus spinosus, as well as a hæmapophysial ridge which divides into two limbs at its distal end.

The plane anterior to the hæmapophysial region (Text-Fig. 10, B) bears a pair of conspicuous transverse processes, which are peculiar in being directed straight downwards from the ventro-lateral borders of the centrum. The neural spine here is reduced to a relatively small pointed projection, while the origin of the hæmapophysial ridge appears like a slight bulging on the lower surface of the centrum.

A little anterior to the plane described above, we come across the articulating anterior region of the vertebra. Here (Text-Fig. 10, C) the processus spinosus is absent and the neural arch roofs the neural space like a rounded dome. The centrum shows no trace of the hæmapophyses, The bases of

the transverse processes, however, are present. They are situated not ventrolaterally to the centrum, but on the outer surface of the neurapophyses distinctly above the centrum. The only parts of the preceding vertebra visible in this section are the backwardly projecting neurapophyses above the neural dome.

A little further ahead we reach the plane of intervertebral foramina (Text-Fig. 10, D). Each spinal nerve comes out through a gap between the neurapophysis of its side and the centrum of the preceding vertebra. The base of the processus spinosus appears in the forms of a pair of horns on the dorsal surface of the neural arch.



TEXT-FIG. 10. Transverse sections through a posterior caudal vertebra of *Enhydrina schistosa*: (A) just anterior to the plane of intervertebral articulation and (B) passing slightly anterior to the hæmapophysial region ( $\times 90$ ).

*h.r.*, hæmapophysial ridge; *d.l.h.*, right limb of the hæmapophysis; *c.*, marrow space; *t.p.*, transverse process. Other abbreviations as in previous figures.

The sections near the tip of the tail differ from the vertebræ of the post-caudal region in two respects. In the first place, the hæmapophysis in them is a single ridge without the distal limbs. Secondly, the bases of the transverse processes extend further forwards even into the articulating anterior region of the vertebra.

## VIII. SUMMARY

The author has made an intensive study of the morphology of snake vertebræ by a careful examination of whole preparations stained with



alizarin and of transverse, frontal and sagittal sections. The more important features discovered are as follows:

(1) The already recognized regions, *precaudal* and *caudal*, of the ophidian vertebral column may be further divided into several sub-regions; viz., the *cervical*, *thoracic*, *lumbar*, *antero-caudal*, *mid-caudal* and *postero-caudal* sub-regions. The characteristics of each sub-region are dealt with.

(2) The general structure of the trunk vertebra in *Serpentes* is described in detail. Amongst the new features discovered may be mentioned the presence of a lateral projection on each side of the prezygapophysis which serves for the attachment of certain muscles and appears to correspond to the *processus mammillaris* of mammals, the differences in the extent and configuration of the *processus spinosus*; the occurrence of two pairs of intervertebral foramina (superior intervertebral and inferior intervertebral) on each side of the vertebral column and a pair of *intravertebral sub-central foramina*.

(3) The trunk vertebrae of *Eryx johni* have been studied in minute detail by means of serial sections. The precise relation of contiguous vertebrae, as well as the nature of intervertebral and vertebro-costal articulations, is described. The supra-central ridge, two supra-central cavities, and the disposition of the marrow-spaces are described for the first time.

(4) The minute anatomy of the trunk vertebrae of *Ptyas mucosus*, *Lycodon aulicus*, *Typhlops braminus* and *Enhydryna schistosa* has been investigated. It is found that the configuration of the vertebra, the structure of the *processus spinosus*, the disposition of the marrow-spaces, the extent of the arching of one vertebra over another, the zygapophysial and zygosphenal articulations, and the occurrence (or otherwise) of the hypocentrum vary from species to species.

(5) The minute anatomy of the caudal vertebrae of *Lycodon aulicus*, *Natrix piscator*, and *Enhydryna schistosa* has been dealt with and the differences between the various sub-caudal regions described.

#### IX. ACKNOWLEDGMENTS

The present investigation was carried out under the direction of Professor Beni Charan Mahendra in the Department of Biology, Birla College, Pilani. My best thanks are due to him for his continuous supervision and painstaking assistance. I am also grateful to Hon. Lt.-Col. S. D. Pande for providing me with the necessary laboratory facilities, and for a great deal of encouragement and interest.

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## SOUTH INDIAN PHYCOMYCETES—II

### Some Little Known Species of *Pythium* Occurring in South India\*

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(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

THE following account is a brief description of some species of *Pythium* collected by the author during the investigation of the *Pythiaceæ* under a scheme financed by the Indian Council of Agricultural Research. The descriptions given are based on the writer's own observations and vary slightly from the original descriptions of the species. For these the reader is referred to Middleton's (1943) excellent monograph of the genus and to Mathew's (1931) handbook "Studies on the genus *Pythium*".

#### I. *Pythium carolinianum* Mathews (*loc. cit.*), p. 71 (Text-Fig. 1)

Hyphæ slender and delicate, measuring  $1.5-4\mu$  in thickness, profusely branched. Sporangia terminal, rarely intercalary, spherical, elongate or limoniform,  $15-30\mu$  in diameter, mostly  $25\mu$ , with a small papilla, proliferous, the secondary sporangia borne either inside or outside the primary sporangia. Zoospores 12 to numerous, delimited in a vesicle formed at the tip of a short evacuation tube, reniform, laterally biciliate, measuring  $8-12\mu \times 6-10\mu$  when swimming and  $8-10\mu$  when encysted, germinating by the production of one to three germ tubes. Sporangia also capable of direct germination. Sexual reproduction not observed. In some 3-4 months' old cultures on slices of boiled carrots in distilled water resting spores (Text-Fig. 1, *L, M.*) similar to those described by Mathews could be observed.

Isolated from water containing vegetable debris, Coimbatore; inoculation trials proved that it could parasitize on *Spirogyra* spp. This species is recorded for the first time outside the United States of America. >

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\* Contribution from the I.C.A.R. School of Mycology, Agricultural Research Institute, Coimbatore.

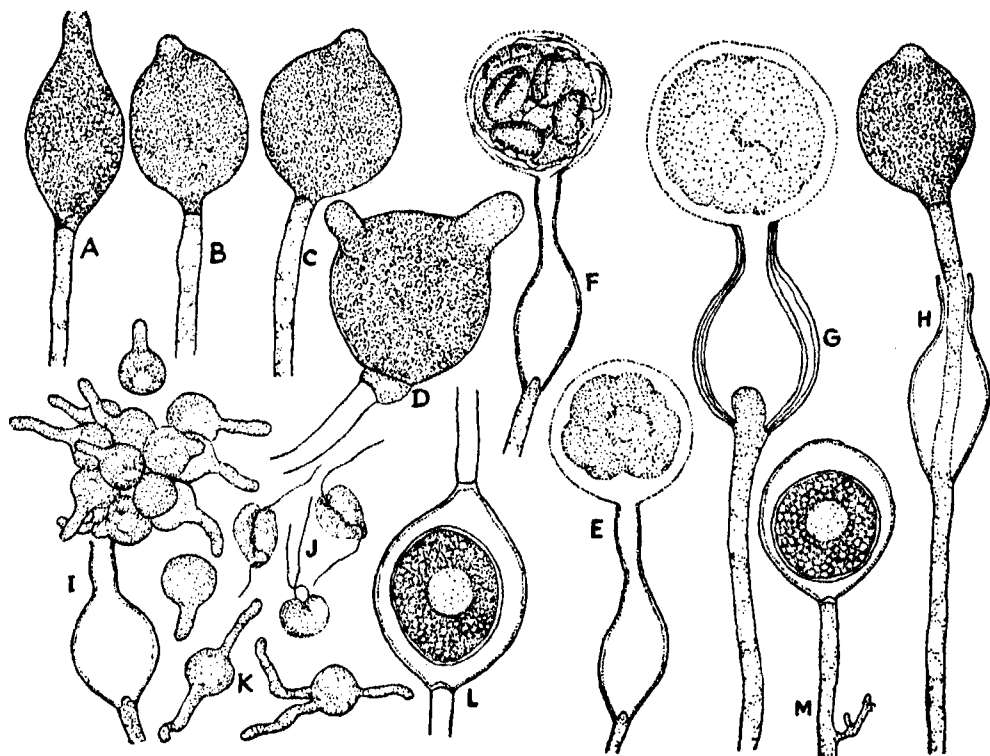


FIG. 1. Asexual reproductive structures of *P. carolinianum* Mathews. All  $\times 680$ .

II. *Pythium paræcandrum* Drechsler, *J. Wash. Acad. Sc.*, **20**, 398-418, 1930  
(Text-Fig. 2)

Hyphae  $2.5-12\mu$  in diameter; curved and clavate appressoria developed in abundance on most culture media. Sporangia spherical, subspherical or ellipsoidal, when spherical  $20-30\mu$  in diameter, when ellipsoidal  $15-40\mu$  long and  $10-30\mu$  wide, terminal or intercalary, germinating by one to several germ tubes. Formation of zoospores not observed. Oogonia subspherical, spherical or barrel-shaped, terminal or intercalary,  $10-30\mu$  in diameter (mean  $24\mu$ ) smooth, occasionally with one or two papillate protuberances. Antheridia 1-4 per oogonium, usually 1-3, monoclinal or diclinal. When monoclinal they may be hypogynous, sessile and adjacent to the oogonium, often including the adjacent segment, pouch-like or crook-necked or rarely terminal on a stalk always arising very near the oogonium. When diclinal they are usually terminal, rarely intercalary, saccate, inflated, crook-necked and sessile or stalked. Oospores aplerotic with a wall upto  $1.5\mu$  thick, containing a single reserve globule and a single refringent body. Oospores measuring  $10-25\mu$  (mean  $19\mu$ ) in diameter.

This rare and interesting species has been reported so far only from *Allium vineale* L. (Drechsler, *loc. cit.*) *Impatiens pallida* Butt., *Sanguinaria canadensis* L. (Drechsler, 1940), *Aloe ciliaris* Haw, and *Aloe variegata* L. (Middleton, *loc. cit.*), all from the U.S.A. The writer isolated it from rotting pods of *Phaseolus vulgaris* L. collected in the New Market, Coimbatore. This is the first record of the species outside the U.S.A..

The most characteristic feature of this species is the diversity of the antheridia in their position and relationship to the oogonium. When diclinous and of remote origin, an antheridium may be saccate, laterally sessile on an axial hypha (Fig. 2, *D*), intercalary (Fig. 2, *E*) or crook-necked, borne terminally on a stalk of varying length (Fig. 2, *F*, *G*, *J*, *M*). In neither instance, however, does it display such variety as when arising in close proximity to the oogonium. The simplest type of a monoclinal antheridium is hypogynous, *i.e.*, an outwardly undifferentiated segment adjacent to the oogonium thrusting its fertilization tube through the septum delimiting the oogonium (Text-Fig. 2, *H*, *I*). Further modification through the production of a lateral outgrowth always arising very near the oogonium permits the protrusion of the fertilization tube through the spherical wall of the oogonium. Here again the lateral portion which is clavate, inflated and crook-necked, may be small in comparison to the segment producing it (Text-Fig. 2, *G*) or when the cylindrical portion is much reduced, may constitute the main bulk of the antheridium (Text-Fig. 2, *B*). This condition approaches cases where the lateral outgrowth is cut off by a septum to function as a sessile antheridium (Text-Fig. 2, *C*, *K*, *L*). Often the antheridium is borne terminally on a stalk of varying length arising always near the oogonium, or a lateral outgrowth may be cut up by a septum to serve as two antheridia in a series. Antheridia thus varying considerably in the manner of origin and shape are found variously associated in many units of the sexual apparatus. This diversity is a characteristic feature of this species and helps to differentiate it from congeneric species such as *P. ultimum* Trow, *P. deBaryanum* Hesse, *P. rostratum* Butler and *P. pulchrum* von Minden.

In his key to species of *Pythium*, Middleton (*loc. cit.*, p. 22) lists this species along with *P. ultimum* and *P. polymorphon* Sideris under the group with "antheridia monoclinal or diclinous but never hypogynous" as distinct from *P. pulchrum* with "typically hypogynous antheridia". As stated above, the simplest type of antheridium in *P. paracandrum* is a hypogynous one, *viz.*, an outwardly undifferentiated segment adjacent to the oogonium which thrusts its fertilization tube through the cross wall

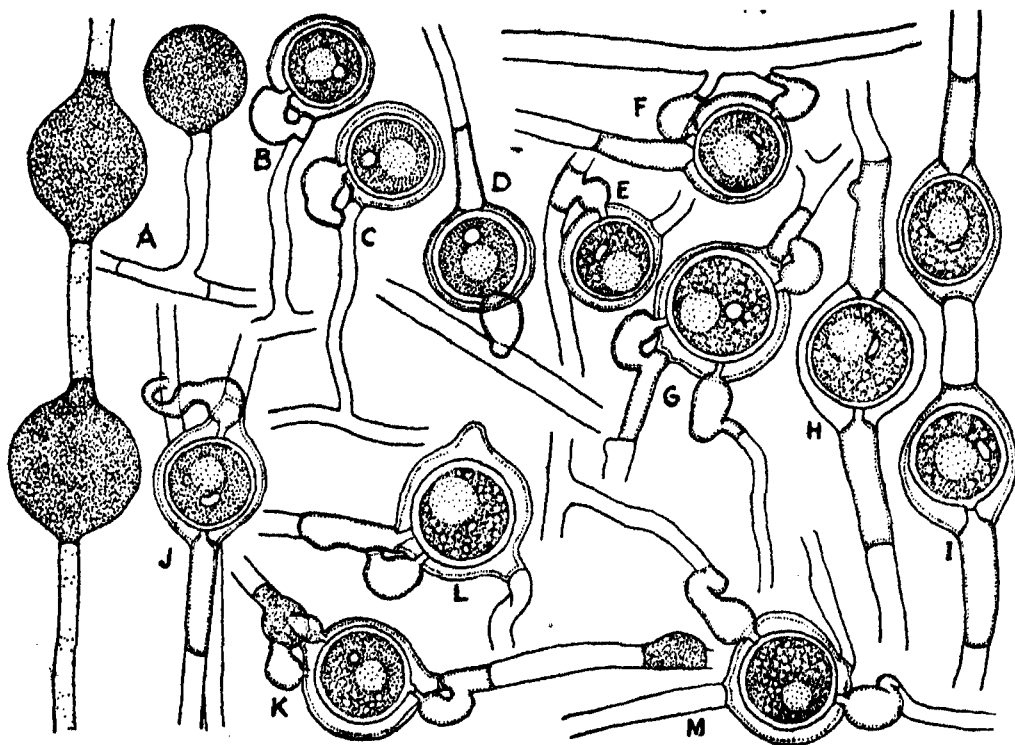


FIG. 2. Asexual and sexual reproductive structures of *P. paroecandrum* Drechsler: A  $\times 480$ , the rest  $\times 680$ .

between it and the oogonium. Middleton himself (*loc. cit.*, p. 6) says that "a hypogynous antheridium is one surmounted by an oogonium; the antheridium is formed within the oogonial stalk or at the apex of the oogonial stalk adjacent to the oogonium, never laterally disposed". Comparison of the figures given by Drechsler (1940) and Middleton (*loc. cit.*, p. 92 A) with Text-Fig. 2, H and I and the "truly hypogynous" antheridia of *P. pulchrum* and *P. rostratum* is enough to show that the simplest types of antheridium in *P. paroecandrum* is also hypogynous. The writer, therefore, feels that *P. paroecandrum* should be considered a species with monoclinal, diclinal and hypogynous antheridia and as such should be placed in a group distinct from *P. polymorphon* and *P. ultimum* which do not possess hypogynous antheridia. The following amendment to Middleton's key is suggested (*cf.*, Middleton, *loc. cit.*, p. 22, line 10).

- Antheridium absent .. .. 39. *P. anguillulae aceti*
- Antheridium present—
- Antheridium typically hypogynous, rarely  
        monoclinous .. .. 40. *P. pulchrum*
- Antheridium monoclinous or diclinous,  
        frequently hypogynous, typically sessile  
        originating near the oogonium .. 41. *P. paroecandrum*
- Antheridium typically monoclinous or di-  
        clinous, never hypogynous .. ..
- Antheridial branch falcate or sigmoid .. 42. *P. polymorphon*
- Antheridial branch not falcate, not sigmoid,  
        antheridium typically sessile, originating  
        immediately adjacent to the oogonium,  
        oospore wall inspissate .. .. 43. *P. ultimum*, etc.

III. *Pythium periplocum* Drechsler var. *Coimbatorensis* var. nov. Text-Fig. 3.

Hyphae 2–9  $\mu$  in diameter, profusely branched, the submerged mycelium being distinguished by intricately ramifying short lateral branches on a straight axial hypha giving a characteristic appearance (Text-Fig. 3, A). Sporangia inflated, filamentous, intercalary, rarely terminal, digitate, often aggregated into massive complexes. Zoospores 50–150, delimited within a vesicle formed at the tip of a long evacuation tube, reniform, laterally biciliate, 8–11  $\mu \times$  9–10  $\mu$  when swimming and 8–10  $\mu$  when encysted, germinating after encystment by one to several germ tubes. In older agar cultures, contents of sporangia retract, forming resting spores with a thin wall and containing several reserve globules and refractive bodies (Text-Fig. 3, B, C) oogonia spherical or subspherical, echinulate, the spines tapering slightly to a blunt apex, terminal, occasionally intercalary, 25–40  $\mu$  (mean 33  $\mu$ ) in diameter, excluding the spines. Antheridia diclinous, one or more, usually one to three, per oogonium; antheridia usually originating on a single stalk, rarely on two stalks. The antheridial cell is oblong clavate, markedly lobed, making ventral contact with the oogonial wall. The antheridia and distal portions of the antheridial stalks extensively and intricately wrapped about the oogonium making it very difficult to trace the relationship of the sexual organs. Oospores aplerotic, spherical, smooth walled, measuring 20–34  $\mu$  (av. 29.5  $\mu$ ) in diameter, with a single reserve globule and a single refractive body.

Isolated from vegetable debris in water, Coimbatore. The species is recorded here for the first time in India. While agreeing in all essential details with the type described by Drechsler, the Coimbatore form differed from the former in one particular, viz., the formation of resting



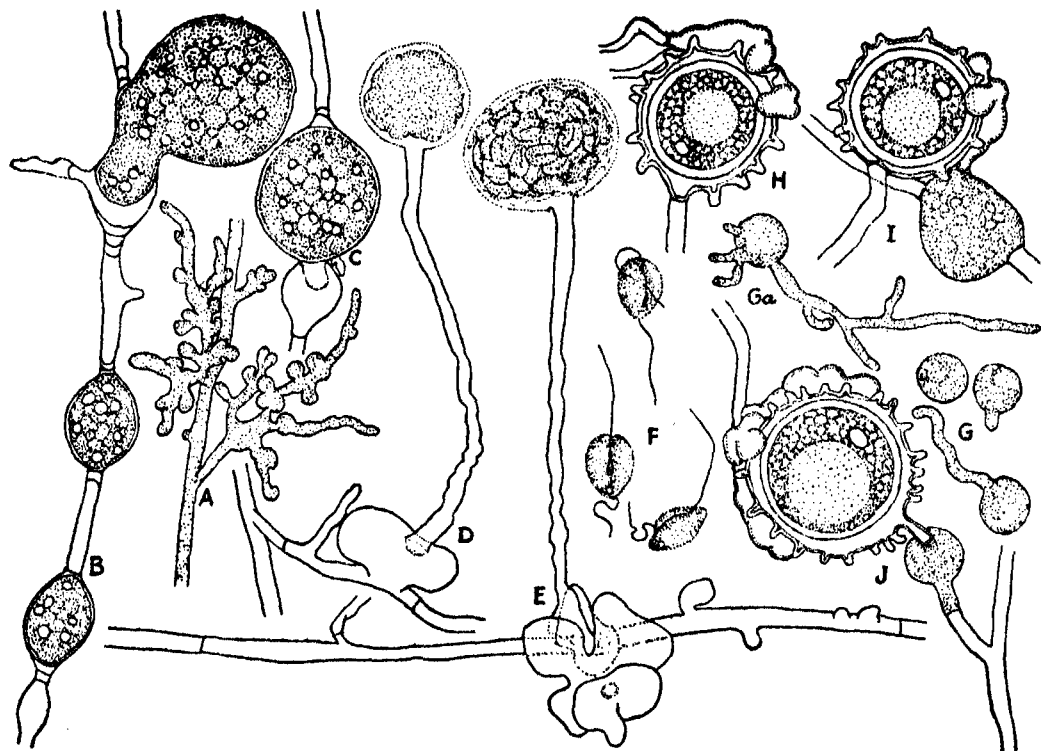


FIG. 3. Asexual and sexual reproductive structures of *P. periplocum* var. *Coimbatorensis* var. nov. A-E  $\times 480$ ; F-J  $\times 680$ .

spores in old agar cultures. These spores (Text-Fig. 3, B, C) were spherical or irregular in shape and appeared to be formed out of sporangia by the retraction of their contents. Mature resting spores did not germinate at all when transferred to distilled water or soil leachate while immature ones readily formed zoospores. The internal organisation of these spores showed a marked similarity to that described for another species of *Pythium*, *P. undulatum* Petersen, by both Dissmann (1927) and Drechsler (1946), though in this case the wall is not quite as thick as that of the resting spores of *P. undulatum*. It is interesting to note that such resting spores have so far been reported for only one species, *P. undulatum*, which like the present form is aquatic. As Drechsler (1946) has remarked, the possibility cannot be ignored that similar reproductive structures may be produced by other species of *Pythium*, especially aquatic forms.  $\times$

Although the present form differs from the type in the production of resting spores, the writer feels that the formation of these spores is to be considered the result of its aquatic habitat and is not a sufficient basis for

considering it specifically distinct from Drechsler's fungus. It is, therefore, presented as a new variety.

*P. periplocum* is at present the only known species of *Pythium* which possesses filamentous sporangia and echinulate oogonia.

IV. *Pythium catenulatum* Mathews (*loc. cit.*), p. 47 (Text-Fig. 4).

Hyphæ slender,  $2-5\mu$  in diameter, profusely branched. Sporangia composed of somewhat inflated hyphæ in conjunction with irregularly swollen outgrowths forming simple or complex aggregations; zoospores 8-100 in a vesicle depending on the size of the sporangium, reniform, biciliate,  $8-10\mu \times 6-7\mu$  when swimming and  $8-10\mu$  when encysted, germinating by 1-3 germ tubes. In addition to sporangia numerous spherical or pyriform asexual reproductive bodies also present; these are terminal or intercalary, single or several in a chain, germinating by germ tubes. Sexual reproduction not observed.

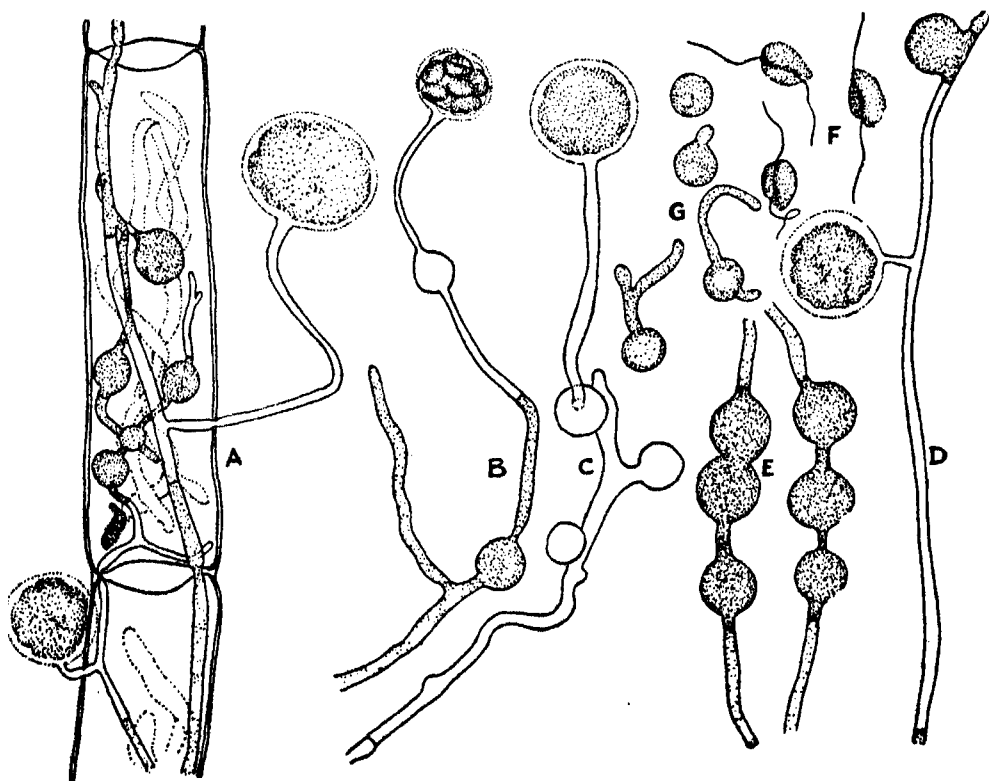


FIG. 4. Asexual reproductive structures of *P. catenulatum* Mathews.  
A-E  $\times 480$ ; F and G  $\times 680$ .

Parasitic on *Spirogyra* spp. in rice fields, Vedapatti, Coimbatore; saprophytic on decaying vegetable matter in water, Coimbatore.

Though all three isolates of this organism collected by the writer did not produce any sexual organs, it is considered identical with *P. catenulatum* on account of the inflated nature of its sporangia which are made up of inflated elements with bud-like lateral outgrowths. In the other two congeneric species which possess spherical asexual reproductive bodies along with filamentous sporangia—*P. perniciosum* Serbinow and *P. afertile* Kanouse and Humphrey—the sporangium is unbranched and not thicker than the parent hypha.

*Pythium catenulatum* has been isolated from vegetable debris in water by Mathews (1931) and Middleton (1943). It is here recorded for the first time outside the United States.

#### SUMMARY

Four species of *Pythium*—*P. carolinianum*, *P. paræcandrum*, *P. periplocum* and *P. catenulatum* are reported for the first time in India. The isolate of *P. periplocum* obtained is considered a new variety as it produces resting spores and is named *P. periplocum* var. *Coimbatorensis* var. nov. The morphological features and peculiarities of each species are described and discussed.

#### ACKNOWLEDGMENTS

It is a great pleasure to acknowledge my indebtedness to Mr. K. M. Thomas, B.A., M.Sc., D.I.C., Government Mycologist, for his kind guidance and criticism throughout the investigation. My thanks are also due to the Indian Council of Agricultural Research for the grant of a research fellowship and to Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc., who was kind enough to go through the manuscript and offer suggestions.

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# ON *CAMPTYLONEMA INDICUM* SCHMIDLE AND *CAMPTYLONEMOPSIS* gen. nov.\*

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[Communicated by Prof. M. O. P. Iyengar, M.A., Ph.D. (Lond.), F.L.S., F.A.Sc.]

*Camptylonema indicum* was first described by Schmidle from Bombay in 1900. Since then the alga has not been recorded again anywhere. An alga which agrees in all respects with *C. indicum* Schmidle was recently collected in Cochin by Prof. K. G. Krishna Rao and sent to Prof. M. O. P. Iyengar, who has kindly placed the material at the disposal of the writer.

This alga was growing among moss plants on the moist walls of a house at Ernakulam in Cochin State, South India, forming an expanded brownish stratum. The filaments are attached to the substratum by their middle portion with the two end-portions growing freely bent upwards (Text-Figs. 1 and 2). These free portions of the filaments were growing densely forming a sort of a felt.

The trichome has a sheath throughout. The portion of the sheath covering the middle region is slightly brownish, while the portions covering the free ends are dark brownish. The sheath is often divergently stratified, but occasionally the layers are nearly parallel. The outermost portions of the sheath is often slightly gelatinized and hyaline.

The filament is broadest in the attached middle portion and is somewhat narrower in the erect portions. In the middle region it is  $13.1-15.7\mu$  broad, occasionally up to  $19.7\mu$  broad, and in the remaining portions ( $7.9-9.2-11.8\mu$  broad. The trichome in the attached middle region is torulose and constricted at the cross-walls, while in the free erect portion it is not constricted or only very slightly constricted. The cells in the attached middle portion are spherical to subspherical and are  $7.9-11.8\mu$  broad and about  $3.9-9.2\mu$  long, and in the free erect portions are cylindrical and are  $3.9-6.6\mu$  broad and  $7.9-18.3\mu$  long. Often the extremities of the trichome are slightly broader and the cells are generally as long as or shorter than broad (Text-Fig. 3). The cell-contents are bluish-green in colour.

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\* From the Department of Botany, University of Madras.

As a rule, a single median heterocyst is found roughly in the middle of the prostrate portion (Text-Fig. 1). This median heterocyst is subspherical or vertically elliptic and generally shorter than broad. Occasionally two heterocysts are found side by side in the middle region (Text-Fig. 2). Heterocysts are also found in the free portions of the trichome (Text-Figs. 3 and 15). In these portions they are cylindrical and up to twice as long as broad. Heterocysts in the prostrate portion are  $7.9\text{--}10.5\mu$  broad and  $3.9\text{--}5.2\mu$  long and in the erect portions about  $6\text{--}7\mu$  broad and  $7\text{--}10.5\mu$  long.

**Branching.**—Branching is generally not very common. But both true and false branches are present. True branches are generally given off from the prostrate middle portion of the filaments, though occasionally they are formed in the erect portions also, while the false branches usually occur in the free erect portions. The writer was able to observe in a single long filament both true and false branches.

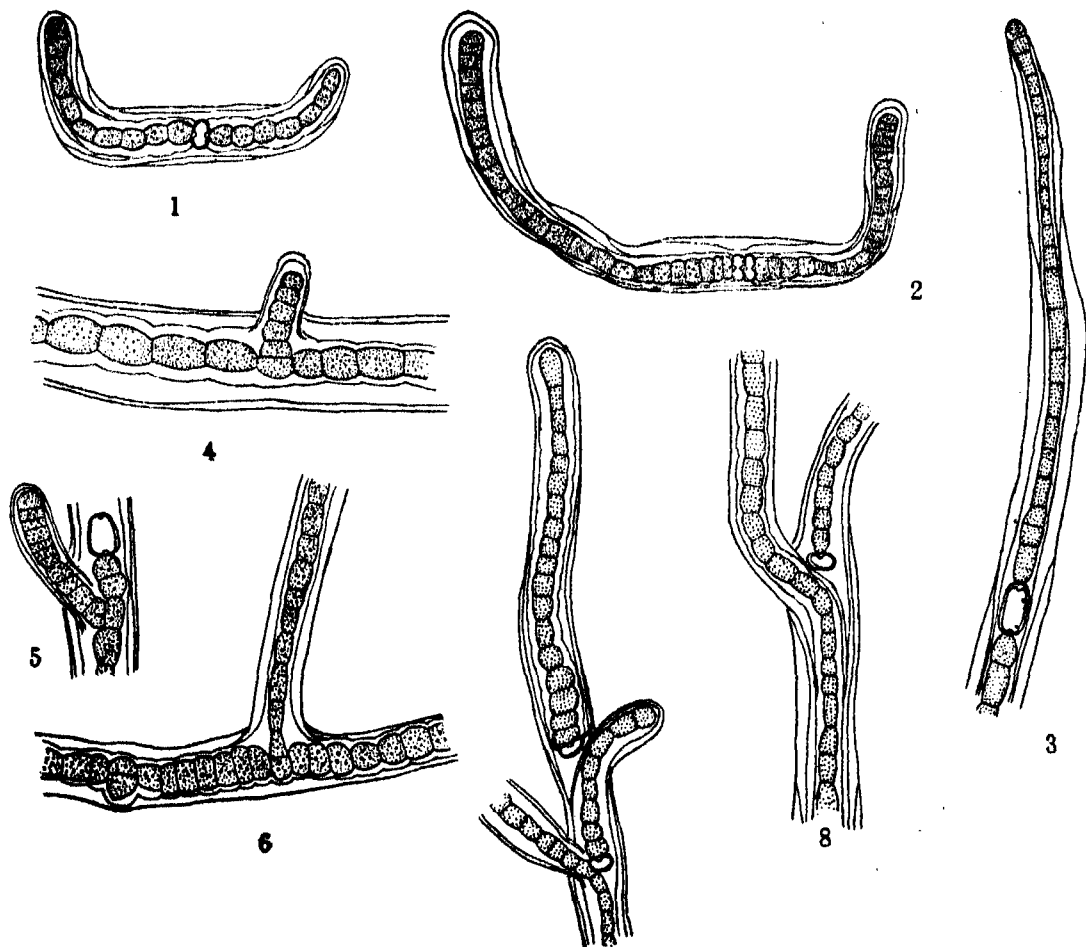
True branches are formed in the following manner. A cell of the prostrate portion enlarges and cuts off a small cell on one side at right angles to the longitudinal axis of the trichome (Text-Fig. 6). This cell by further divisions forms a short lateral branch which pierces through the parent sheath and comes out (Text-Fig. 4). As it grows out it secretes its own sheath (Text-Figs. 4–6).

False branches are generally given off singly as in *Tolypothrix* (Text-Figs. 7 and 8). In these cases, usually the cell by the side of the false branch becomes converted into a single-pored heterocyst. Geminate false-branches were not observed by the author.

**Hormocysts.**—From the terminal free portions of the filaments are cut off small portions of the trichome, which are completely surrounded by a thick lamellated sheath. These short portions are described by Schmidle as 'pseudohormogonia', and correspond to Borzi's 'hormocysts'.\* These pseudohormogonia (hormocysts) are formed in the following manner. The apical portion of a trichome (about 4–12 cells long) increases slightly in breadth. This portion soon shows distinct constrictions at the cross-walls. This end portion gets cut off from the main trichome by the formation of a biconcave disc of intercellular substance or through the degeneration of an intercalary cell (Text-Figs. 9 and 10). It then secretes a thick lamellated, brownish sheath all round itself (Text-Fig. 11). The pseudohormogonium

\* Fritsch (1927, p. 447) gives the following definition for *hormocysts*: "In some Scytonemataceæ and Stigonemataceæ short hormogonia, which are completely enclosed in a thick-walled and stratified sheath, are formed (Borzi's *hormocysts*).<sup>\*</sup> In the present paper, Schmidle's term *pseudohormogonia* is used to avoid confusion.

(hormocyst) gets finally liberated by the disintegration of the parent sheath. Usually a single pseudohormogonium is formed at a time, but occasionally two pseudohormogonia may be formed one behind the other.

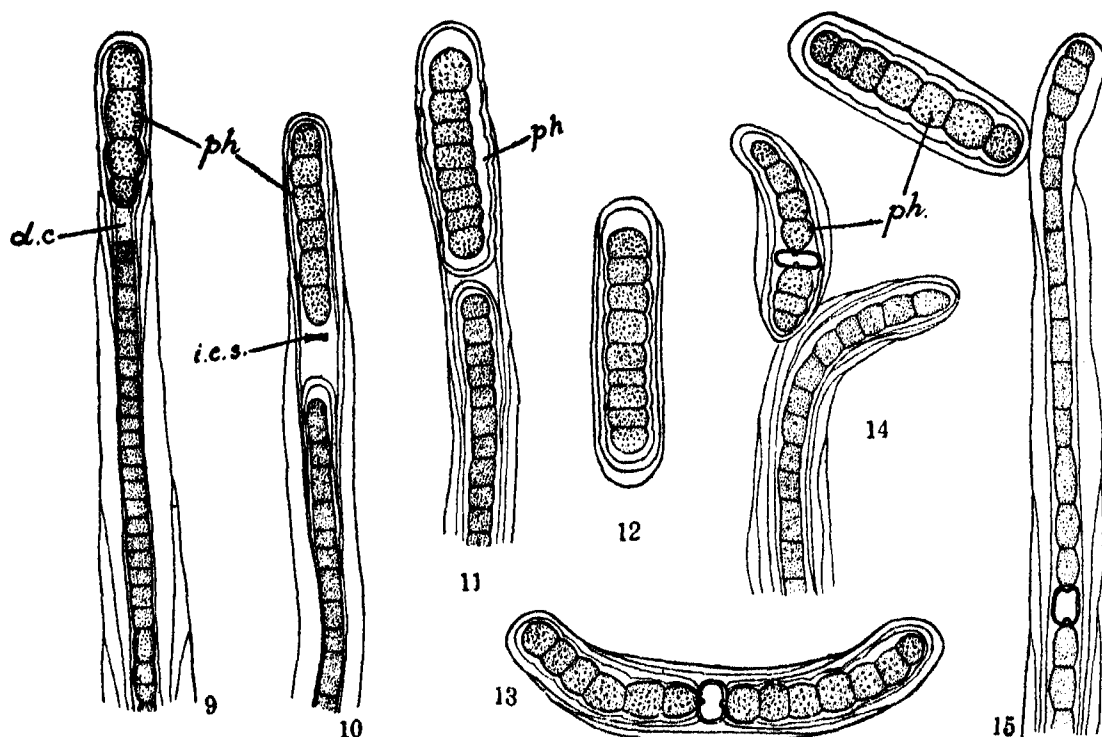


TEXT-FIGS. 1-8.—*Camptylonema indicum* Schmidle.—Fig. 1. An young filament showing the characteristic crescent-shaped habit with a single median heterocyst. Fig. 2. An young filament showing the characteristic crescent-shaped habit with two median heterocysts side by side. Fig. 3. End portion of a filament with an intercalary heterocyst. Figs. 4-6. Portions of filaments showing true branches. Figs. 7-8. Portions of filaments showing single false-branches by the side of one-pored heterocysts. Figs. 1-8  $\times 475$ .

The pseudohormogonium after liberation germinates. During germination, one of its central cells becomes transformed into a heterocyst (Text-

Fig. 13). Then the filament grows further, and its two ends bend upwards and grow erect.

Very occasionally, the pseudohormogonium is not liberated from the parent filament. In such cases, the portion of the parent filament below the pseudohormogonium grows past it bending sideways, while the pseudohormogonium germinates attached to one side of the parent filament (Text-Figs. 14, 15).



TEXT-FIGS. 9-15.—*Campylonema indicum* Schmidle.—Fig. 9. End portion of a filament showing the pseudo-hormogonium (hormocyst) (*ph*) being cut off by a dead cell (*d.c.*). Fig. 10. End portion of a filament showing the pseudohormogonium (hormocyst) being cut off by a biconcave disc of intercellular substance (*i.c.s.*). Fig. 11. End portion of a filament showing a pseudohormogonium which has secreted a sheath all round. Fig. 12. A liberated pseudohormogonium. Fig. 13. A young germinating pseudohormogonium with a median heterocyst. Figs. 14-15. End portions of filaments showing pseudohormogonia germinating attached to the parent filament. Figs. 9-15  $\times 725$ .

No spores have been observed in the alga.

#### SYSTEMATIC POSITION

According to Schmidle's diagnosis, *Campylonema indicum* has crescent-shaped filaments which are attached to the substratum by their middle por-

tion with the two end-portions of the filament growing freely upwards and possesses both true and false-branches. Pseudohormogonia (hormocysts) are formed at the apices of the filaments. The present alga has all these features. Its filaments are crescent-shaped and are attached to the substratum by the middle portion. It has both true and false branches. And pseudohormogonia (hormocysts) are formed at the ends of the filaments. In dimensions also it agrees fully with Schmidle's *Camptylonema indicum*. The writer, therefore, considers that the present alga is the same as Schmidle's *Camptylonema indicum*.

Schmidle places the genus *Camptylonema* under the Stigonemaceæ. This he does evidently on account of the presence of true branches in the alga. But Forti (in De Toni, *Sylloge Algarum*, 1907, p. 540) placed it under the Scytonemaceæ, though he retained in the generic description, the occurrence of true branches in addition to false branches. Ghose (1920) described an alga from Lahore which possessed crescent-shaped filaments like those of *Camptylonema indicum* Schmidle and false-branches. Though true branches were not found in this alga, he described it as a new species of *Camptylonema*, *Camptylonema lahorensis*. He, like Forti, referred the genus *Camptylonema* to the Scytonemaceæ. Geitler (1932, p. 705), doubting the presence of true branches in the genus, modified Schmidle's original diagnosis of the genus by deleting the occurrence of true branching in his description of the genus. He, however, adds finally that, in case true branching should be found in *C. indicum*, then it should be included under the Stigonemataceæ and that under *Fischerella* (with a query). Now that clear cases of true branches have been observed by the writer in *C. indicum*, the genus *Camptylonema* should now be retransferred to the Stigonemataceæ as suggested by Geitler. The writer, however, does not agree with Geitler's suggestion that it should be included under *Fischerella*. The writer considers it best to retain the genus, *Camptylonema* as defined by Schmidle (1900).

In this connection, Geitler (1932) states again, that in case true branching should be found in *Camptylonema indicum* then Ghose's alga, *Camptylonema lahorensis*, (which shows only false branching, but no true branching), should be placed in a new genus. This is a most reasonable suggestion. Ghose's alga may therefore now be placed in a new genus which may be called *Camptylonemopsis*. This genus may be included under Microchaetaceæ. In case true branching should later on be found in Ghose's Lahore alga, it may then be retransferred to *Camptylonema*.

Hollerbach (1934) described an alga from Russia which he called *Camptylonema Danilovii*. It resembles Ghose's Lahore alga in having the



crescent-shaped filaments with occasional false-branching but without any true branching. This alga also may be placed under *Camptylonemopsis*.

Three new algæ resembling Ghose's Lahore alga were collected by Prof. Tyengar from South India and have been placed by him at the writer's disposal. The writer proposes to include these three algæ also under *Camptylonemopsis*. A brief account of the three new algæ is given below.

*Camptylonemopsis pulneyensis* sp. nov.

This alga was found in a collection of algæ from one of the pools at the side of the lake at Kodaikanal in the Pulneys, in South India.

The plant mass is somewhat gelatinous and consists of a large number of filaments growing densely aggregated. These filaments show the characteristic crescent-shaped growth of the genus with a prostrate middle portion and the free end portions growing bent upwards (Text-Fig. 16). The filaments are fairly long and are  $7.9-11.8 (-13.1) \mu$  broad.

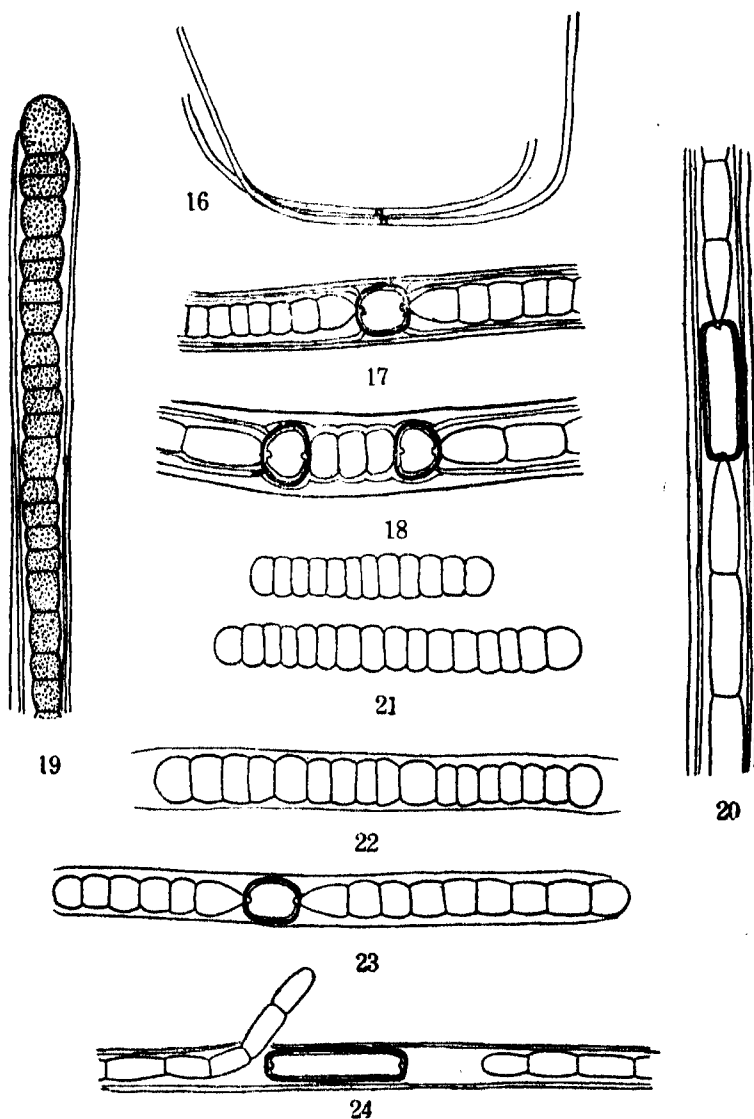
The filament is provided with a thick sheath which is hyaline and is somewhat lamellated. The trichome in the middle portion is slightly broader than in the side portions. And the cells of the middle regions are spherical, subspherical or barrel-shaped and measure  $5.2-9.2 \mu$  broad while in the remaining side portions they are cylindrical and longer than broad and measure  $3.9-6.6 \mu$  in breadth and up to  $20 \mu$  in length. At the extreme ends of the trichome the cells are generally broader and are slightly shorter than broad (Text-Fig. 19).

Usually a single heterocyst is present in the middle of the filament. The heterocyst is spherical to subspherical and measures  $7.9-10.5 \mu$  in breadth. Sometimes two such spherical heterocysts are found in the middle region separated by a few vegetative cells (Text-Fig. 18). Intercalary heterocysts are found throughout the length of the filament. These are slightly cylindrical and are  $5.2-7.9 \mu$  broad and  $7.9-19.65 \mu$  long (Text-Fig. 20).

False-branching appears to be extremely rare. Only in one case, a small false branch was seen arising by the side of a dead two-pored heterocyst (Text-Fig. 24).

Hormogones are often cut off from the ends of filaments. These hormogones, when they are liberated, are devoid of any sheath (Text-Figs. 21-22). Usually one of the middle cells of the hormogone becomes a heterocyst (Text-Fig. 23). When the hormogone grows, its ends grow curving upwards in crescent-shaped manner.

No spores were observed in the alga.



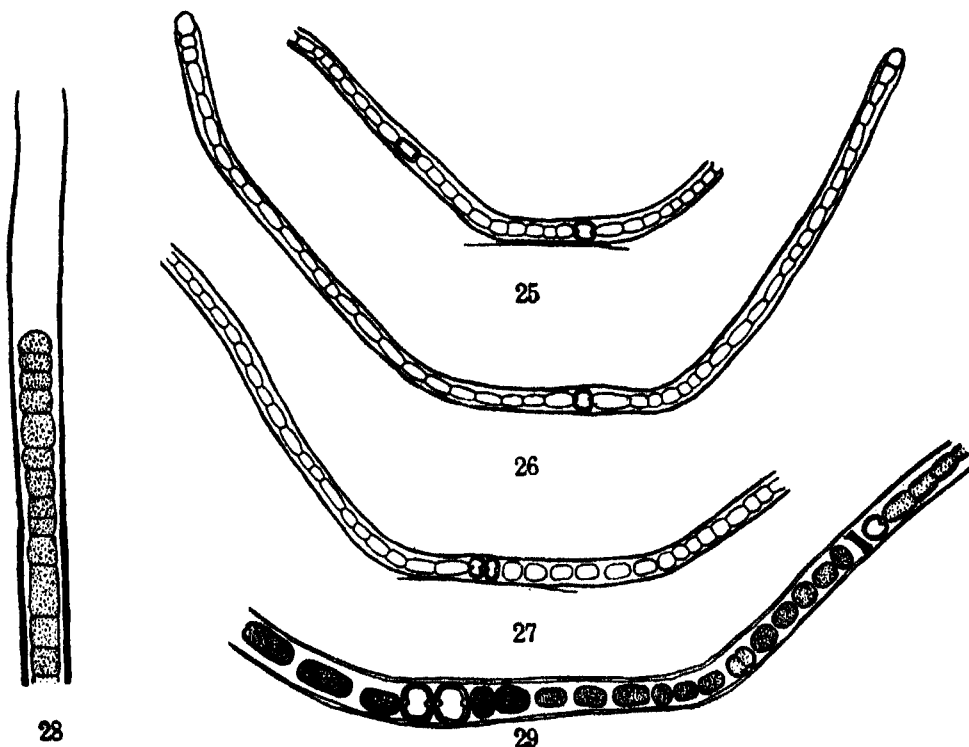
TEXT-FIGS. 16-24.—*Camptylonemopsis pulneyensis* sp. nov. Fig. 16. Crescent-shaped filaments (diagrammatic). Fig. 17. Portion of a filament showing median heterocyst. Fig. 18. Portion of a filament showing two median heterocysts separated by three vegetative cells. Fig. 19. End portion of a filament. Fig. 20. Portion of a filament with an intercalary heterocyst. Fig. 21. Hormogones. Fig. 22. Hormogone with a thin sheath. Fig. 23. A germinating hormogone with a single median heterocyst. Fig. 24. Portion of a filament showing a false-branch by the side of an intercalary heterocyst. Fig. 16  $\times 75$ ; Figs. 17-23  $\times 725$ ; Fig. 24  $\times 475$ .

This alga, while resembling *Camptylonemopsis lahorensis* (Ghose) comb. nov. in having crescent-shaped filaments, differs from it in dimensions and also in the fact that it is an aquatic form, while the Lahore alga is a terrestrial one. Again, false-branching in the present alga is extremely rare or nearly absent. The alga may, therefore, be described as a new species and called *Camptylonemopsis pulneyensis* sp. nov.

*Camptylonemopsis minor* sp. nov.

This alga was found growing epiphytically on the filaments of the previous form, *Camptylonemopsis pulneyensis*, in the same pool at Kodaikanal.

The filaments of this alga show the characteristic crescent-shaped growth (Text-Figs. 25-27), but are much shorter than *Camptylonemopsis*



TEXT-FIGS. 25-29. *Camptylonemopsis minor* sp. nov.—Fig. 25. Portion of a filament showing the characteristic crescent-shaped habit showing a single median heterocyst and another heterocyst in the side portion. Fig. 26. A full filament showing the crescent-shaped habit with a single median heterocyst. Fig. 27. A portion of filament showing the crescent-shaped habit with two median heterocysts. Fig. 28. End[portion of a filament. Fig. 29. Portion of a filament with a series of spores. Figs. 25-27  $\times 475$ ; Figs. 28-29  $\times 725$ .

*pulneyensis*. The filament is  $3.9-7.8\mu$  broad and is throughout constricted at the cross-walls. The cells are spherical to subspherical near the median heterocyst, but, in the remaining portions, the cells are barrel-shaped and are  $3.9-9.2\mu$  long. At the ends of filaments, however, the cells are slightly broader and shorter than broad (Text-Fig. 28).

Generally a single median heterocyst is found in the middle of the filament (Text-Figs. 25-26). This median heterocyst is  $3.9-6.6\mu$  broad. Sometimes two such spherical heterocysts are seen together in the middle region (Text-Figs. 27 and 29). Intercalary heterocysts are present in the other portions of the filament also (Text-Fig. 25). These intercalary heterocysts are cylindrical and are up to twice as long as broad and measure  $3.9-6.6\mu$  broad and  $(3.9) 5.2-10.5 (-13.1)\mu$  long.

No branching was found in the alga.

Plenty of spores are formed in this alga. Spore-formation begins close to a heterocyst on either side of it and extends further outwards. These spores are quadrate to cylindrical in shape and are generally found in series which may be often interrupted by vegetative cells (Text-Fig. 29). The spores have a brownish outer wall and are  $3.9-6.5\mu$  broad and  $5.2-10.5\mu$  long.

This alga differs from *Camptylonemopsis lahorensis* (Ghose) comb. nov. in dimensions and also in its aquatic habit. It agrees, however, with *Camptylonemopsis lahorensis* in forming spores in series. This alga appears to be a new species and may be called *Camptylonemopsis minor* sp. nov.

*Camptylonemopsis Iyengarii* sp. nov.

This alga grows epiphytically on the filaments of *Sirogonium* sp., growing in water which was trickling over the soil at the foot of the hill at Vandalur, a place twenty miles south of Madras.

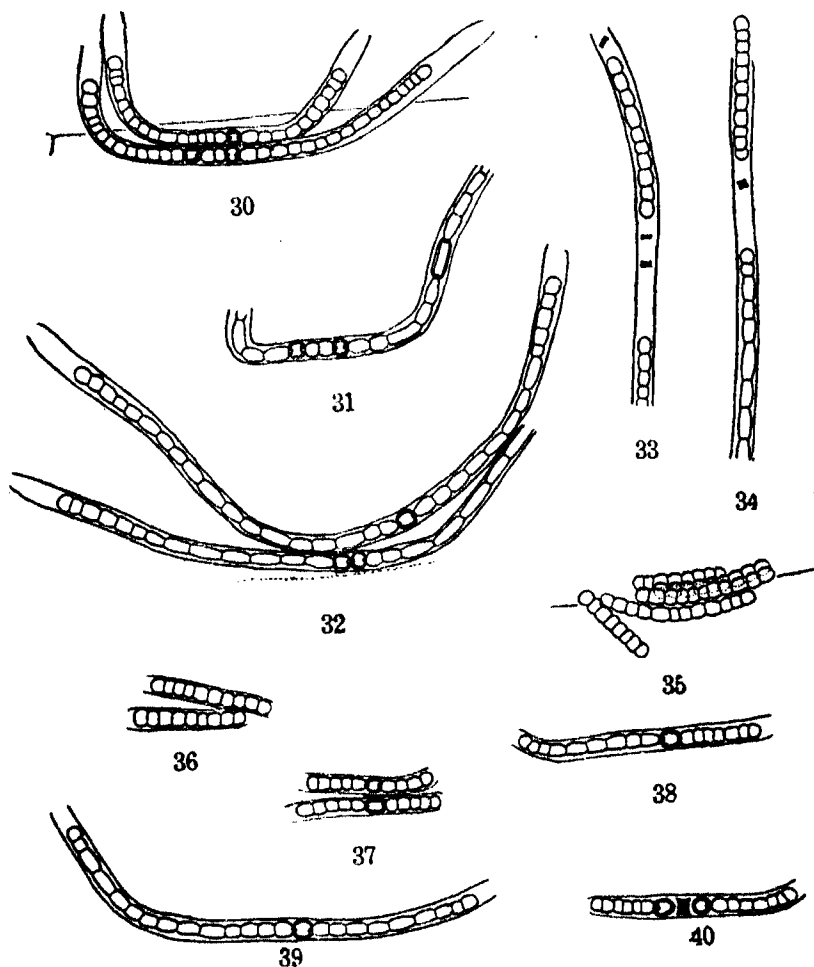
The filaments of the alga showed the characteristic crescent-shaped bending of *Camptylonemopsis* and were attached to the host alga by their middle portion with the two side portions growing freely upwards (Text-Fig. 30). The filaments are  $5.2-6.6\mu$  broad and have a thin hyaline and homogeneous sheath.

The filaments are unbranched. No case of branching was observed though numerous specimens were examined.

The trichome is deeply constricted at the cross-walls and is broadest in the attached middle portion and narrower in the side portions. The extreme ends, however, are slightly broader. The cells in the middle region are roughly spherical and are  $3.9-5.2\mu$  broad. The cells in the side

portions are barrel-shaped and are longer than broad and measure  $2.6-3.9\ \mu$  broad and  $5.2-10.5\ \mu$  long.

Heterocysts are formed both in the middle and in the side portions. The heterocysts formed in the middle portion are generally spherical (Text-Fig. 30) and are  $3.9-6.6\ \mu$  broad; but the heterocysts in the side



TEXT-FIGS. 30-40. *Camptylonemopsis lyengarii* sp. nov.—Fig. 30. Two young filaments showing the characteristic crescent-shaped habit, one of them with a single median heterocyst and the other with two median heterocysts separated by two vegetative cells. Fig. 31. Portion of a filament with two median heterocysts separated by two vegetative cells and also showing an intercalary heterocyst in the erect portion. Fig. 32. Portions of filaments, one with a single median heterocyst and the other with two median heterocysts side by side. Figs. 33-34. End portions of filaments showing hormogones. Figs. 35-40. Stages in the germination of the hormogones. Figs. 30-40  $\times 475$ .

portions are generally cylindrical and up to two to three times as long as broad (Text-Fig. 31). These latter are  $3.9-5.2\ \mu$  broad and up to  $15.7\ \mu$  long.

Hormogones are formed terminally and are cut off from the main trichome by the formation of biconcave discs of intercellular substance (Text-Figs. 33 and 34). They are liberated from the ends of the filament. Several of these young hormogones were found growing on the host filaments. They at first possess no sheath (Text-Fig. 35). In the later stages, a definite sheath is seen round each one of them (Text-Fig. 36). These young hormogones grow horizontally attached to the host plant for some time and soon their end portions bend and grow upwards. All the stages from the young hormogones to the fully-formed crescent-shaped filaments were found on the host alga.

In the young hormogones all the cells are vegetative but soon one of the middle cells becomes a heterocyst (Text-Figs. 37-39). In the fully developed filament sometimes two heterocysts are seen side by side in the middle (Text-Fig. 32). These two heterocysts are two-pored, suggesting that two adjacent cells in the middle of the trichome have become converted into heterocysts. In some cases, both the heterocysts are one-pored, the single pore being found on the side away from the intercellular disc (Text-Fig. 40). This suggests that the trichome was broken up into two portions by a disc of intercellular substance and that the cells on either side of the disc become a heterocyst, the pore being formed only on the side on which the heterocyst was in contact with the remaining portion of the trichome (*cf. Camptylonemopsis lahorensis*, Text-Fig. 44). Sometimes two heterocysts are found in the middle of the filament separated by one or more cells (Text-Figs. 30, 31). In these cases both the heterocysts are two-pored suggesting that two separate cells in the middle of the filament have developed into two intercalary heterocysts.

No spores were found in the alga.

This alga shows a certain amount of resemblance to Hollerbach's *Camptylonemopsis Danilovii* (Hollerbach) comb. nov. (= *Camptylonema Danilovii* Hollerbach), but differs from it in dimensions and in general appearance. It differs from the previously known species in its growing nearly always on *Sirogonium* and in its semi-aquatic habitat. This alga appears to be new. The writer has much pleasure in naming this new alga *Camptylonemopsis Iyengarii* sp. nov., after Professor M. O. P. Iyengar, his revered Professor.

#### SYSTEMATIC POSITION OF *Camptylonemopsis*

The genus *Camptylonemopsis* shows a resemblance to *Aulosira* in being unbranched though occasionally having false-branches and in forming spores

in series. It differs from *Aulosira* in its being attached by its middle portions with its two side portions growing bent upwards.

It shows a certain amount of resemblance to *Microchæte* also in its growing attached to a substratum though it differs from it in the mode of attachment. In *Microchæte* one end of the filament is attached to the substratum while the other end grows freely upwards, while in *Camptylonemopsis* the middle portion of the filament is attached to the substratum, while the two end-portions grow freely upwards. It also resembles *Microchæte* in the formation of spores in series. In *Microchæte* the terminal cell of the short attached prostrate portion becomes a heterocyst, while in *Camptylonemopsis* a cell in the middle of the attached prostrate portion becomes a heterocyst. The *Microchæte*-condition could easily be derived from *Camptylonemopsis* by the suppression of the free upward growth on one side of the germinating hormogone through its end-cell on that side becoming converted into a heterocyst instead of one of the middle cells becoming converted into a heterocyst as in *Camptylonemopsis*. In this connection it may be mentioned that in *Camptylonemopsis* frequently two heterocysts are often seen side by side separated by an intercalary disc in the prostrate portion (Figs. 40 and 44). In this condition the alga has the appearance of two *Microchæte* filaments facing each other and growing in opposite directions.

*Camptylonemopsis* would, thus, appear to be related to both *Aulosira* on the one hand and *Microchæte* on the other and to form a natural connecting link between the two genera. The genus may be placed in the Microchætaceæ between *Aulosira* and *Microchæte*.

#### DIAGNOSIS OF THE GENUS *Camptylonemopsis* GEN. NOV.

Filaments bent more or less like a crescent, with a median heterocyst and with two ends of filaments growing upwards, simple or rarely false-branched; true branches absent. Trichome forming a single row of cells. Heterocysts intercalary or at the base of a false-branch. Hormogonia present. Hormocysts not known. Spores formed in series.

Key to the species of *Camptylonemopsis* gen. nov.

1. Aquatic
  - A. Filament 7·9–13·1  $\mu$  broad .. .. *C. pulneyensis*
  - B. Filament 3·9–7·9  $\mu$  broad .. .. *C. minor*
2. Semi-aquatic or on wet soil
  - A. Epiphytic
    - Filament 5·2–6·6  $\mu$  broad .. .. *C. Iyengarti*
  - B. Not epiphytic
    - i. Trichome 6–9  $\mu$  broad .. .. *C. lahorensis*
    - ii. Trichome 2–3·3 (–3·7)  $\mu$  broad .. .. *C. Danilovii*

DESCRIPTION OF THE SPECIES

1. *Camptylonemopsis pulneyensis* sp. nov.

(Text-Figs. 16–24)

Plant mass small, light blue-green in colour; filaments bent into a crescent-shape,  $7.9\text{--}13.1\mu$  broad; sheath firm, thick, lamellated, and hyaline; unbranched or rarely with false-branches; true branches absent; trichome in the middle region  $5.2\text{--}9.2\mu$  broad, narrower in older portions (rarely even  $3.9\mu$  broad), and in the side portions  $3.9\text{--}6.6\mu$  broad; apices slightly broader than the portion below and rounded; trichome generally constricted at the cross-walls; cells shorter or longer than broad,  $5.2\text{--}20\mu$  long; heterocysts intercalary, median heterocysts spherical and  $7.9\text{--}10.5\mu$  broad, in the other portions cylindrical,  $5.2\text{--}7.9\mu$  broad and  $7.9\text{--}19.6\mu$  long; hormogones present.

*Hab.*—Among other algæ in a pool near lake, Kodaikanal.

2. *Camptylonemopsis minor* sp. nov.

(Text-Figs. 25–29)

Filaments short, flexuous,  $3.9\text{--}7.9\mu$  broad; sheath thin, hyaline, firm without any distinct lamellation; branching not seen; trichome with one or two median heterocysts, constricted at the cross-walls,  $2.6\text{--}5.2\mu$  broad; apices of trichome slightly broader, rounded; cells spherical or barrel-shaped,  $3.9\text{--}9.2\mu$  long; heterocysts usually intercalary nearly spherical when median,  $3.9\text{--}6.6\mu$  broad, in other portions cylindrical and up to twice as long as broad; spores formed in series close to the heterocysts,  $3.9\text{--}6.6\mu$  broad and  $5.2\text{--}10.5\mu$  long.

*Hab.*—Epiphytic on *Camptylonemopsis pulneyensis* in a pool near lake, Kodaikanal.

3. *Camptylonemopsis Iyengarii* sp. nov.

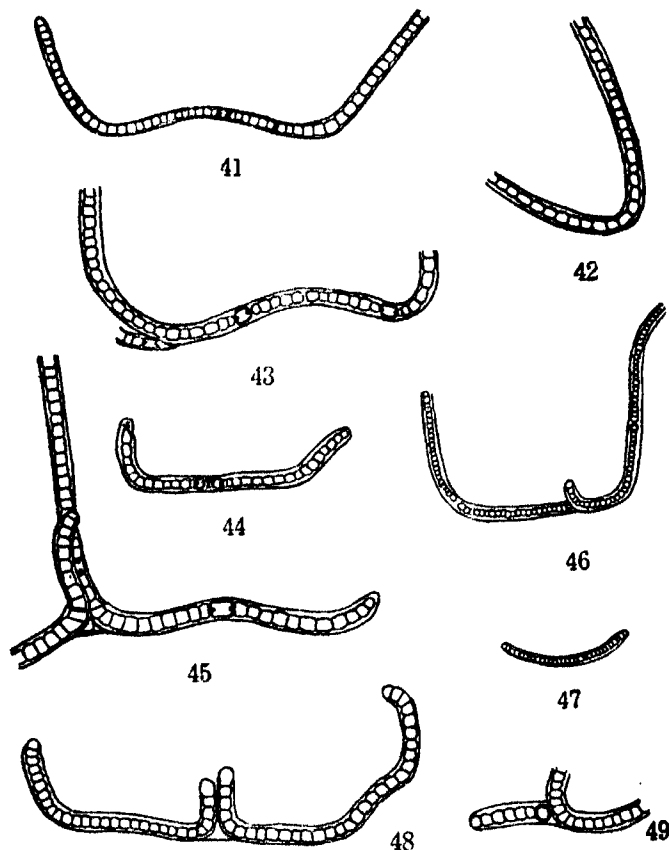
(Text-Figs. 30–40)

Filaments epiphytic on *Sirogonium* filaments, crescent-shaped with a flat attached middle region and lateral erect portion,  $5.2\text{--}6.6\mu$  broad; sheath thin, hyaline, homogeneous; false-branching not seen; trichome torulose ( $2.6\text{--}$ )  $3.9\text{--}5.2\mu$  broad; apex of the trichome slightly broader, rounded; cells spherical, barrel-shaped or sometimes cylindrical,  $3.9\text{--}5.2\mu$  broad in the middle region and near the apex,  $2.6\text{--}3.9\mu$  broad and  $5.2\text{--}10.5\mu$  long in the rest of the trichome; heterocysts intercalary, median heterocysts



nearly spherical,  $(3.9-5.2-6.6\mu)$  broad, in other portions  $3.9-5.2\mu$  broad and up to  $15.7\mu$  long; hormogones present; spores not seen.

*Hab.*—Epiphytic on filaments of *Sirogonium* sp., on moist soil, Vandalur.



TEXT-FIGS. 41-45. *Camptylonemopsis lahorensis* (Ghose) comb. nov. (after Ghose).—Fig. 41. A typical filament showing the incomplete sheath, median heterocyst and intercellular substance. Fig. 42. A filament showing a chain of spores. Fig. 43. A filament showing many heterocysts and a single false-branch at the base of one of them. Fig. 44. A filament showing two median heterocysts. Fig. 45. An old filament showing the thick sheath and geminate false branches. Fig. 41  $\times 150$ ; Figs. 42-43 and 45  $\times 225$ ; Fig. 44  $\times 210$ ;

TEXT-FIGS. 46-49. *Camptylonemopsis Danilovii* (Hollerbach) comb. nov. (after Hollerbach).—Fig. 46. A filament with a single false-branch and two heterocysts. Fig. 47. An young filament. Fig. 48. A filament with geminate false-branch. Fig. 49. Single false-branch. Figs. 46-47  $\times 300$ ; Figs. 48-49  $\times 550$ .

4. *Camptylonemopsis lahorensis* (Ghose) comb. nov.<sup>1</sup>

(=*Camptylonema lahorense* Ghose, *The New Phytol.*, **19**, 35-39, figs. 1-6, 1920)

(Text-Figs. 41-45)

Thallus woolly, bright bluish-green, or bluish brown, terrestrial, partly embedded in mud and partly above it; sheath inconspicuous, thin and hyaline in the embedded region and firm, thick and lamellose, lightly adhering and brown in the exposed portion; filaments curved in a more or less semicircular manner, up to  $1\frac{1}{4}$  mm. in length; the trichome bluish-green,  $6-9\mu$  broad, slightly constricted at the joints; rarely pseudo-branched, pseudobranches given off singly or in pairs; cells isodiametric or a little longer than broad, transverse walls scarcely conspicuous in the older filaments; heterocysts median or found at intervals throughout the length of the filament, rectangular or ellipsoidal,  $12-21\mu$  long and  $7-9\mu$  wide; spores  $7-11\mu$  long and  $5-7\mu$  wide, formed in a chain within the sheath, epispodium brown and smooth; cell-contents coarsely granular.

*Hab.*—On damp lawns and waste grounds at Lahore, India.

5. *Camptylonemopsis Danilovii* (Hollerbach) comb. nov.<sup>2</sup>

(=*Camptylonema Danilovii* Hollerbach, *Acta Inst. Bot. Acad. Sci., URSS.*, Series II, fasc. 2, p. 40, fig. I, 9-12, 1934)

(Text-figs. 46-49)

Filament  $3.3-3.7\mu$  broad, solitary or several, constantly semi-circularly curved, ascending; simple or rarely false-branched; branches single or geminate; trichome in the middle  $2-3.3\mu$  broad, at the apices somewhat broadened,  $3.3-3.7\mu$  broad, constricted at the cross-walls; cells barrel-shaped, usually as long as broad, rarely shorter,  $2.1-4.2\mu$  long; contents homogeneous, light blue-green; sheath thin, often diffuent at the apices, in the middle slightly broader; heterocysts rare, intercalary, nearly rectangular or short-oval,  $2.8-3.3\mu$  broad and  $2.1-3.3\mu$  long.

*Hab.*—In superficial strata of clay soil (pH 6.3).

SUMMARY

The genus *Camptylonema* was established by Schmidle on the type-species, *Camptylonema indicum*, which had been collected from Bombay

<sup>1</sup> This description is adapted from Ghose (1920).

<sup>2</sup> This description is after Hollerbach (1934).

The genus was placed by him under the Stigonemaceæ, since both true and false-branches were found by him in the alga. Forti, Ghose and Geitler, doubting the occurrence of true branching in the alga, transferred the genus to the Scytonemaceæ. The type-species, *C. indicum*, which was not recorded again since Schmidle described it, was recently recorded by the writer from Cochin in South India. Undoubted cases of true branching are found in this alga in addition to false-branches. Since true branches are found in the alga, the genus is now retransferred to the Stigonemataceæ.

A new genus, *Camptylonemopsis*, is created to include species like *Camptylonema lahorensense* Ghose and *Camptylonema Danilovii* Hollerbach, which possess crescent-shaped filaments, but do not show true branching. The new genus, *Camptylonemopsis*, is placed under the Michrochætaceæ.

Three new species of *Camptylonemopsis* from South India, viz., *C. pulneyensis* sp. nov., *C. minor* sp. nov. and *C. Iyengarii* sp. nov., are described in the paper.

In conclusion, the writer expresses his great indebtedness to Professor M. O. P. Iyengar for his constant guidance and valuable help throughout the course of this work. The writer's sincere thanks are also due to the authorities of the University of Madras for the award of a Studentship during the tenure of which this investigation was carried out.

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# NEUROMUSCULAR TRANSMISSION IN FROG'S UNSTRIATED MUSCLE

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CONDITIONS affecting neuromuscular transmission in frog's striated and cardiac muscle are well known. There is no corresponding record of such observations on frog's unstriated muscle. In the present paper are described certain conditions which affect the transmission of nerve impulse across the neuromuscular junction in frog's unstriated muscle.

## EXPERIMENTAL

The nerve smooth muscle preparation was as described previously, using circular strips (Singh and Singh, 1947 *a*). Arrangement was made to stimulate the muscle either directly or through the nerve.

## RESULTS

*Effect of fatigue.*—It is well known that the neuromuscular junction in striated muscle is more susceptible to fatigue than the muscle itself. The same is found in unstriated muscle. If the latter is stimulated continuously through the nerve, fatigue is very rapid, the tension subsiding in a couple of minutes. If now the current is switched on to the muscle, the latter contracts powerfully (Fig. 1). This suggests that neuromuscular transmission in both the muscles must be similar.

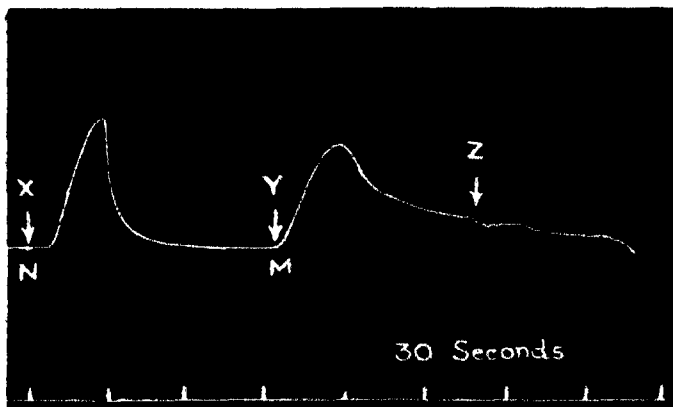


FIG. 1. Frog stomach muscle. The muscle was stimulated through nerve from X to Y, and then directly from Y to Z.

If the muscle fatigues to acetylcholine (1 in 10,000) stimulation through the nerve produces contraction showing that acetylcholine could not be a transmitter. The same happens if the muscle does not respond to acetylcholine, that is, naturally fatigued. Sometimes adrenaline causes contraction. If the muscle fatigues to adrenaline, then stimulation through the nerve causes contraction, showing that adrenaline could not be a transmitter. Most commonly, in 95 per cent. of the preparations used, the muscle was naturally fatigued or irresponsive to adrenaline, but the neuromuscular transmission was normal. These experiments show that neither adrenaline nor acetylcholine are chemical transmitters, so that transmission must be electrical (Singh and Singh, 1947 a).

Stimulation through the nerve diminishes the contractions produced by acetylcholine and potassium. The same is produced by alternating current (Singh, 1938); this is understandable as the properties of the contraction produced through nervous stimulation are similar to those produced by alternating current (Singh and Singh, 1947 a). Frequent stimulation through nerves reduces the sensitivity of the muscle to acetylcholine, adrenaline and potassium, just as it happens if the muscle is frequently stimulated by alternating current (Singh and Singh, 1946). These experiments account for the increased sensitivity to neuro-hormones after denervation.

*Effect of calcium.*—Calcium is necessary for transmission of nerve impulse in striated muscle. If the nerve smooth muscle preparation is repeatedly washed with calcium-free saline, then neuro-muscular transmission is abolished in about 2-3 hours, the response to direct stimulation being the same or bigger (Fig. 2). Thus calcium is necessary for neuromuscular transmission in unstriated muscle. The optimum amount of calcium required is that contained in the saline (Singh, 1939), but if the tissue is washed with calcium-free saline for about 4 hours, then the amount of calcium required may be one and a half times as much in the beginning. This suggests that intracellular calcium is a factor responsible for transmission, as this will be depleted by repeated washing with calcium-free saline, and more calcium will be required in the saline to raise the level of intracellular calcium to its previous value in a given time.

Excess of calcium above the normal limits is depressant, more to the neuromuscular junction than to the muscle. Thus the neuromuscular reaction is abolished in calcium content of saline, which is 3 to 4 times the normal. Similarly excess of other divalent cations such as magnesium, strontium and barium are more toxic to the neuromuscular junction than

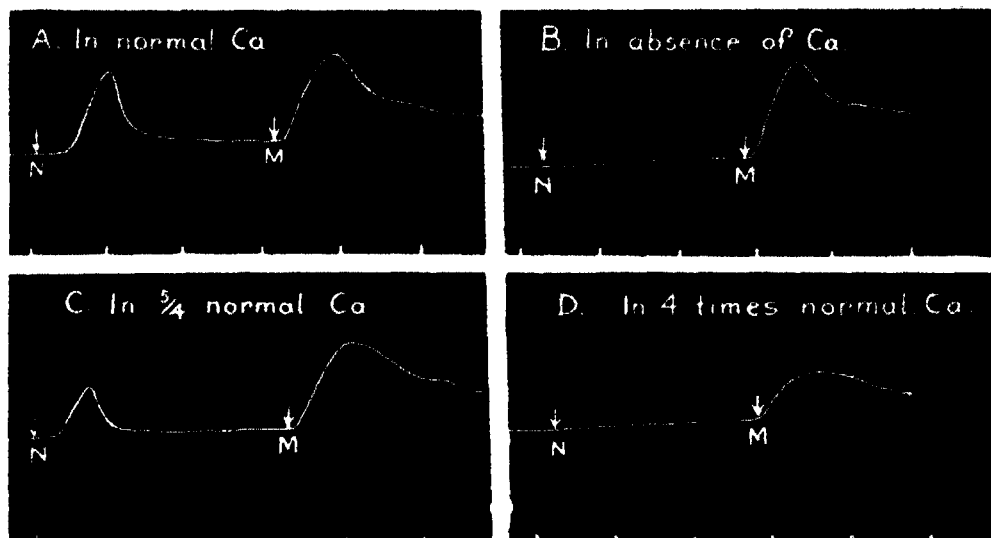


FIG. 2. Frog stomach muscle. Effect of calcium. Muscle stimulated through nerve at N for 30 secs. and directly at M for 30 secs. Timing 30 seconds.

to the muscle. It is thus possible that the fatigue at the neuromuscular junction is due to liberation of calcium (Singh, 1944).

*Effect of potassium.*—In the absence of potassium, neuromuscular transmission fails (Fig. 3). The optimum amount of potassium required is that found in the saline (Singh, 1939), but if the preparation is washed with potassium-free saline for about 2 hours, then the optimum concentration of potassium required is about 30 per cent. of normal. This shows that the effect of potassium is dependent upon its ratio without and within the cells, as washing of the preparation in potassium-free saline would diminish the intracellular potassium, and the original ratio of potassium without and within the cells would now require less potassium without.

The preparation also adapts to potassium; if potassium above 30 per cent. of normal is depressant, then the depression diminishes with time, so that, ultimately the normal concentration of potassium is necessary. The depressant action of potassium is overcome by calcium or by increase of osmotic pressure of the saline (Singh, 1939). Excess of ammonium also has a depressant action. Ammonium is known to have a curariform action in striated muscle.

*Effect of sodium chloride.*—Diminution of the electrolyte content of the saline by replacing the sodium chloride with sucrose, diminishes the neuromuscular more than the muscular response. In the electrolyte-free medium, the neuromuscular response is abolished but not the direct one.

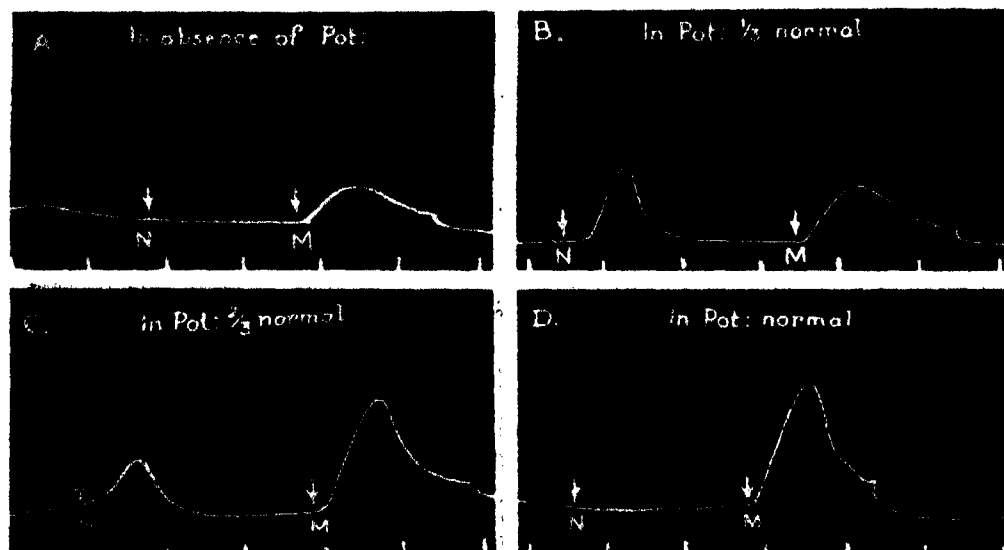


FIG. 3. Frog stomach muscle. Effect of potassium.

Replacement of the sodium with lithium also depresses neuromuscular transmission. Excess of hydrogen ions (pH 5) has similar action.

*Effect of anions.*—Replacement of the chloride of the saline with bromide, nitrate, iodide, thiocyanate is more depressant to the neuromuscular than to the direct response, the effect increasing in the order  $\text{Cl} < \text{Br} < \text{NO}_3 < \text{I} < \text{SCN}$ .

*Effect of drugs.*—Adrenaline, eserine, acetylcholine, nicotine, strychnine in toxic doses (1 in 10,000) are more depressant to the indirect than the direct response. Curare (saturated solution of impure substance) has similar action, which appears to be a non-specific one.

*Effect of anoxia.*—Neuromuscular transmission is paralysed before the muscle as a result of oxygen lack or treatment with cyanide; glucose produces partial relief (Singh and Singh, 1948).

*Spontaneous contractions.*—The muscle may not be excitable through nerves, but the spontaneous contractions are usually present. This shows that spontaneous contractions are myogenic and not neurogenic. Similarly the response to electric current is myogenic. The third contraction produced by direct current (Singh and Singh, 1947 *b*) is also myogenic.

## DISCUSSION

The neuromuscular junction in unstriated muscle is more susceptible to toxic action of substances than the muscle itself. No specific action of

any substance causing paralysis of the neuromuscular junction in unstriated muscle has been found. Calcium and potassium play an important role in transmission.

#### SUMMARY

1. The neuromuscular junction in frog's unstriated muscle is more susceptible than the muscle to fatigue, toxic action of substances and oxygen lack.

2. Calcium and potassium are necessary for neuromuscular transmission.

3. Spontaneous contractions and those produced by electric current are myogenic.

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## ADDITIONS TO FUNGI OF MADRAS—V

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### 26. *Guignardia nilagiriaca* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, yellowish green, orbicular, without a definite margin; *spermagonia* red, amphigenous, innate, deep-seated, ostiolate, with numerous, hyaline, slender, rod shaped, spermatia, which in mass appear reddish; *spermagonia*  $118-158 \times 122-160 \mu$  and spermatia  $4-12 \times 1 \mu$ . *Perithecia* of same size as *spermagonia*, black, ostiolate; *asci* cylindric-clavate, hyaline, 8-spored,  $52-62 \times 8-12 \mu$ ; *ascospores* uniseriate or irregular, oblong, hyaline, one-celled,  $8-12 \times 4-6 \mu$ ; paraphyses absent.

On living leaves of a Papilionaceous plant, Kallar (Coimbatore), 7-VIII-1947, T. S. Ramakrishnan and K. Ramakrishnan.

Maculae amphigenae, orbiculares, flavo-virentes; *spermagonia* rubra, amphigena, innata, ostiolata,  $118-158 \times 122-160 \mu$ ; spermatia numerosa, hyalina, tenua, baculo-formia, massa spermatiorum rubra,  $4-12 \times 1 \mu$ , *Perithecia* nigra, ostiolata; *asci* cylindric-clavati, hyalini, octosporiati,  $52-62 \times 8-12 \mu$ ; *ascosporidia* oblonga, hyalina, unicellata  $8-12 \times 4-6 \mu$ ; paraphyses absunt.

In vivis foliis Papilionaceae species, Kallar (Coimbatore), 7-VIII-1947, T. S. Ramakrishnan et K. Ramakrishnan.

The leaf spot is irregularly studded with numerous pink or red *spermagonia* in the early stages. As the spot enlarges fresh *spermagonia* develop along the margin of the spot while those in the centre turn black. Thus a mixture of red and black fructifications can be seen on the same spot. The contents of the young *spermagonia* appear red with innumerable rod-shaped spermatia. These are pushed out of the *spermagonia* in masses, as bright red tendril-like outgrowths. Side by side with these are older fructifications in which several multicellular filamentous structures project from the base of the loculus. In these the basal cells are stouter while those at the apex are attenuated resembling trichogynes. Further the apical attenuated portion does not easily stain with eosin. The tips of these filaments project out through the ostiole. The exact nature and function of these structures

are being studied. Mixed with these filaments are numerous hyaline outgrowths from the lining of the cavity. At a later stage when the fructifications have turned black, asci can be noticed originating from the base of the cavity. Typical paraphyses are absent but remnants of the abovementioned filamentous outgrowths can be seen near the ostiole (Plate II *d, e, f*).

The isolated formation of unilocular perithecia without typical paraphyses and the presence of hyaline one-celled ascospores indicate that the fungus belongs to the genus *Guignardia*. The genus *Guignardia* is used here in the sense of *G. Bidwellii* (Ellis) Viala and Rav. (Chardon, *et al.*, 1940).

27. *Catacauma elaeocarpi* Ramakrishnan, T. S. and K. sp. nov.

Stromata epiphyllous, black, shiny, more or less circular, up to 0.5 cm. in diam., scattered, centre raised, almost intra-epidermal, multiloculate; locules ostiolate, round or slightly flattened; *asci* fusiform, 8-spored, hyaline,  $62 \times 18 \mu$  ( $51-71 \times 17-20 \mu$ ); *ascospores* irregularly arranged, hyaline, one-celled, oblong,  $16.5 \times 6.6 \mu$  ( $13-19 \times 5-9 \mu$ ); paraphyses filiform.

On living leaves of *Elaeocarpus munroi* Mast. Coonoor (Nilgiris), 10-X-1947, T. S. Ramakrishnan.

Stromata epiphylla, nigra, micantia, circiter orbicularia, usque 0.5 cm. diam., sparsa, intraepidermia, multiloculata; *asci* fusiformi, clavati, octosporiati,  $62 \times 18 \mu$  ( $51-71 \times 17-20 \mu$ ); *ascosporidia* oblonga, hyalina,  $16.5 \times 6.6 \mu$  ( $13-19 \times 5-9 \mu$ ); paraphyses filiformes.

In vivis foliis *Elaeocarpi munroii* Mast. Coonoor (Nilgiris), 10-X-1947, T. S. Ramakrishnan.

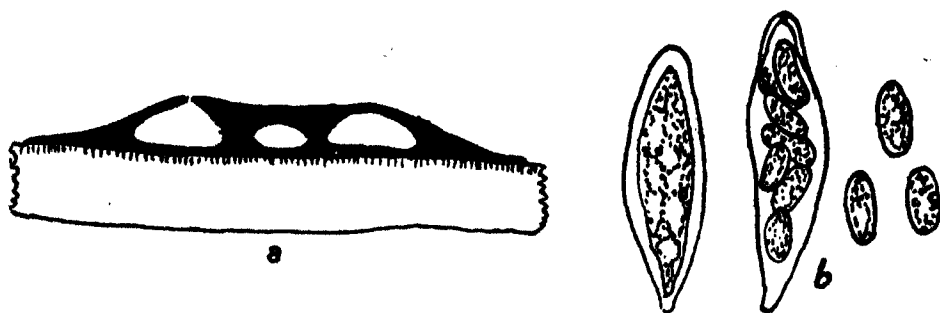


FIG. 1. *Catacauma elaeocarpi*.—*a*. Section of leaf showing stroma and perithecia (diagrammatic). *b*. asci and ascospores  $\times 400$ .

In sections of the leaves the stroma is present as a dark mass between the palisade tissue and the epidermis and also filling the latter. In some places extensions of plates of hyphae can be recognised in the palisade tissue. However a sharp dark distinctive limit is visible above the palisade cells

denoting the stroma. Consequently the fungus has to be included in the *Scirrhiineæ* in the genus *Catacauma*.

*Phaeodothiopsis elaeocarpi* (Racib.) Theiss. and Syd. has been described by Theissen and Sydow (1915) on *E. angustifolia* from Java. Though this resembles *Catacauma* to some extent, its spores are two celled and brown in colour, and therefore different from the fungus under study. Four species of *Phyllachora* have also been described by the same authors on Tiliaceous hosts. But these differ in the position and size of the stromata, from the present fungus. No species of *Catacauma* has been recorded on this genus and therefore the fungus under study is considered to be a new species.

28. *Ustilago shiraiana* P. Henn.

Saccardo, P. A., *Syll. Fung.*, 1902, 16, 369.

Butler, E. J., and Bisby, G. R., *Fungi of India*, 1931, 50.

Pattersen, F. W., and Charles, V. K., *Phytopath.*, 1919, 6, 351-56.

On young shoots of *Arundianaria wightiana* Nees. Tiger Hill, Ootacamund (Nilgiris), 20-V-1947, T. S. Ramakrishnan and T. V. Subramanian.

The sori are formed at the ends of young shoots. They are caulicolous and enclosed between the sheaths of the older leaves. The sheaths of the young leaves in the sorus region are sometimes destroyed. No covering of fungal origin is evident in the sorus. The spore mass is dark brown and pulverulent. The spores are globose to subglobose or elliptic, smooth,  $7-10 \times 6-10 \mu$ . The spores easily separate from one another and are tawny olive in colour with granular contents.

All the shoots in one clump were affected while in a neighbouring clump there was no infection indicating the systemic nature of infection. This is a new host for this smut.

29. *Ustilago sporoboli-tremuli* Ramakrishnan, T. S. and K. sp. nov.

Sori caulicolous 2-3 mm. long, enclosed inside leaf-sheaths at the ends of young shoots, without a covering of fungal tissue; spore mass black, semi-agglutinated and semi-powdery; spores separate easily, cinnamon brown, subglobose, epispore up to  $1 \mu$  in thickness, punctate when examined under oil immersion,  $16 \times 15 \mu$  ( $14-19 \times 14-17 \mu$ ).

On shoots of *Sporobolus tremulus* Kunth. Chettipalayam, Coimbatore, 17-VII-1934, N. Kitchi Naidu.

Soris cauliculis, 2-3 mm. longis, massa sporarum nigra, pulverulenta; sporis cinnamomeo brunneis, subglobosis,  $16 \times 15 \mu$  ( $14-19 \times 14-17 \mu$ ), episporio usque  $1 \mu$  crasso, subtiliter punctate,

In caulibus *Sporoboli tremuli* Kunth. Chettipalayam, Coimbatore, 17-VII-1934, N. Kitchi Naidu.

Clinton (1904) has recorded three species of *Ustilago*, viz., *U. vilfa*, *U. sporoboli*, and *U. hypodites* on this genus from America. Of these the first two are ovaricolous with prominently verrucose or tuberculate spores. *U. hypodites* has sori surrounding the internodes but the spores measure  $4-7\mu$ . It is obvious that the smut under study is different from the above species. Zundel (1938) has described *U. Schlechteri* P. Henn. on *Sporobolus* sp. from Natal. But this smut affects the inflorescence and has smaller spores ( $7-10\mu$ ).

30. *Uracium nothopegiae* Ramakrishnan, T. S. and K. sp. nov.

*Pycnia*, *uredia* and *telia* not known; *aecia* uredinoid, hypophyllous, subepidermal, deep seated; *aeciospores* pedicellate, oblong, reniform or angular, one celled, light brown in colour,  $15 \times 8\mu$  ( $12-18 \times 6-10\mu$ ), finely verrucose.

On living leaves of *Nothopegia* sp. near Naduvattam (Nilgiris), 21-V-1947, T. S. Ramakrishnan and T. V. Subramanian.

*Pycnia*, *uredia* et *telia* ignota; *aecia* uredinoidea, hypophylla, subepidermate, profunde immersa; *aeciosporidia* pedicellata, oblonga, reniformia, vel angularia, leviter brunneo colore, cum una cellula, subtiliter verrucosa,  $15 \times 8\mu$  ( $12-18 \times 6-10\mu$ ).

In vivis foliis *Nothopegiae* sp. Naduvattam (Nilgiris), 21-V-1947, T. S. Ramakrishnan et T. V. Subramanian.

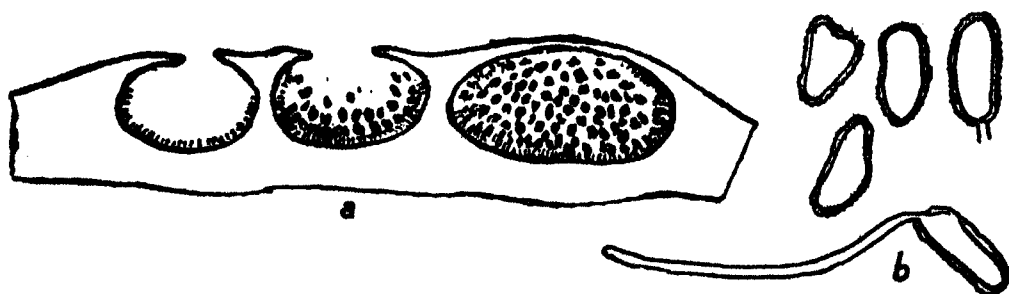


FIG. 2. *Uracium nothopegiae*.—a. Section of leaf showing sori (diagrammatic). b. *aeciospores* ( $\times 400$ ).

This rust produces witches broom formations at the ends of shoots or from sides of branches. The hypertrophied growth (Plate I, a) consists of several swollen branches with crowded, reduced and thickened leaves. The shoots are one and a half to twice the thickness of healthy ones of the

same age. The hyphæ of the rust penetrate the tissues of the host, being inter-cellular in the cortex and medullary rays; they are found in the vessels also. The normal leaves are green in colour; but those on the witches broom are light pinkish yellow. There is gradual diminution in the size of the leaves from the base to the apex of the affected branches, those at the apex being reduced to scale leaves.

The æcia are found on the lower surface of the leaves of the witches broom distributed all over the surface (Plate II, *b*). Each æcium is deep seated and the epidermis forms a protruding dome-like arch. The cavity so formed is filled with spores. At a later stage the epidermis splits open forming an oval to round pore in the centre of the dome through which the spores escape. Peridium is absent and the spores are not formed in chains. They are stylosporic. The fructification resembles the æcia described by Arthur (1934) as occurring in the form genus *Uræcium*. Consequently it is included in this genus for the present. No rust has been recorded on this host and this rust is described as a new species, *U. nothopegia*.

31. *Puccinia thomasiana* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, circular or irregular, isolated or confluent, light to dark brown on the upper side. *Pycnia*, *æcia* and *uredia* not known. *Telia* cauliculous, hypophyllous, closely crowded, pulvinate, warm sepia coloured, rounded, sub-epidermal, surrounded by the remnants of the epidermis which form a ring. *Teliospores* two-celled, clavate to elliptic, slightly constricted at the septum, ochraceous tawny,  $60 \times 22 \mu$  ( $43-77 \times 14-24 \mu$ ), wall smooth, apex rounded or bluntly pointed, thickened up to  $14 \mu$ ; germ pore one in each cell, apical and below the septum; pedicel hyaline, or lightly coloured, persistent, up to  $80 \mu$  in length.

On living leaves and stem of *Ocimum gratissimum* L. Anamalais, 3-X-1922, N. Kitchi Naidu.

Maculæ amphigenæ, orbiculares vel irregulares, singulares vel confluentes, fulvo vel fusco colore superficie superiore. *Pycnia*, *æcia* et *uredia* ignota; *telia* caulicola, hypophylla, dense aggregata, pulvinata, fusco-rubro colore, rotundata, sub-epidermate. *Teliosporidia* cum duobus cellulis, clavata vel cylindrica, medio leviter constricta, ochraceo-nigricante colore,  $60-22 \mu$  ( $43-77 \times 14-24 \mu$ ) paries levis, apice rotundato vel obtuso, usque ad  $14 \mu$  densatus, foramen germinationis unum in una quaque cellula; pedicellus hyalinus vel leviter coloratus, persistens, usque ad  $80 \mu$  longus.

In ramis et foliis vivis *Ocimi gratissimi* L. Anamalais, 3-X-1922, N. Kitchi Naidu.

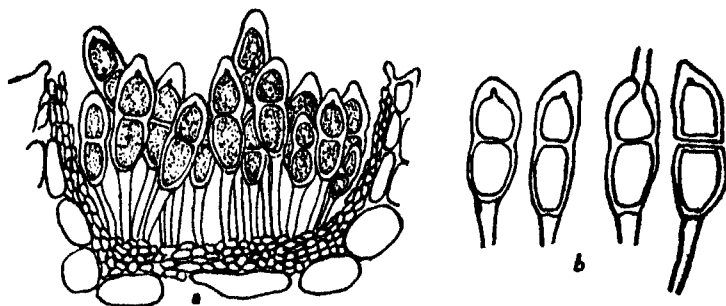


FIG. 3. *Puccinia thomasiiana*.—a. Section through telium ( $\times 200$ ), b. teliospores ( $\times 250$ ).

The telia are in crowded circular groups. They are developed in deep seated cuplike depressions lined by a plectenchymatous tissue of small cells. A ring formed by the remnants of the host tissue which has been burst through is clearly visible round the telium. When scrapings from old telia were examined it was found that several of the teliospores separated into one-celled portions along the septum. Teliospores are capable of immediate germination (*in situ*) as shown by the remnants of the basidia emerging through the germ pores in some of the teliospores.

Two species of *Puccinia* have been recorded on *Ocimum*. *P. ocimi* has been described by Doidge (1926) on *O. suave*, and *O. americanum* from South Africa. Thirumalachar (1941) has observed *P. leiocarpum* on *O. adscendens* from Mysore. Butler and Bisby (1931) have mentioned *Aecidium ocimi* on leaves of *O. cannum* from Koilpatti, Madras. Thirumalachar considered this to be the æcial stage of *P. leiocarpum*. It must however be mentioned that the host plant in this collection has been erroneously named *O. cannum*. It is *O. adscendens*, and the record of the rust on *O. cannum* should be revised. The rust under study is not *P. leiocarpum* as the teliospores are much bigger. They are even longer than those of *P. ocimi*. Doidge has stated that the telia of *P. ocimi* are cauliculous and hypophyllous often interspersed with the æcia. In the present fungus æcia have not been observed. The telia are both cauliculous and hypophyllous. The infected portion of the stem becomes swollen and is studded with closely arranged telia. The comparative measurements of the teliospores of the two rusts are given below.

	Teliospores	Thickness of apex
<i>P. ocimi</i> (Doidge)	40–60 $\times$ 16–24 $\mu$	up to 8.5 $\mu$
<i>Puccinia</i> on	43–77 $\times$ 14–24 $\mu$	11.5 (10–14 $\mu$ )
<i>O. gratissimum</i>	(mean 60 $\times$ 22 $\mu$ )	

The longer teliospores with thicker apices and the absence of æcia, show that this rust is different from *P. ocimi*. It is described as a new species, *P. thomasiana* in honour of Mr. K. M. Thomas, Government Mycologist, Coimbatore.

32. *Puccinia tweediana* (Speg.) Ramakrishnan, T. S. and K. comb. nov.

*Pycnia* not known; rust spot amphigenous, circular, dark brown on the upper surface; *æcia* hypophyllous, crowded in the spot, cupulate, with orange contents; peridial cells polygonal, thick-walled hyaline, prominently verrucose,  $25 \times 18 \mu$  ( $17-29 \times 13-21 \mu$ ); *æciospores* subglobose, catenulate, light yellow, wall smooth or very finely verrucose,  $17.5 \times 14 \mu$  ( $14-19 \times 10-18 \mu$ ); *uredia* absent; *telia* rare, mixed with the *æcia*, hypophyllous, sub-epidermal, appearing as chocolate coloured raised pustules; *teliospores* stipitate, two celled, clavate, apex obtuse, thickened up to  $8.5 \mu$ , slightly constricted at the septum,  $47 \times 17 \mu$  ( $32-56 \times 12-20 \mu$ ), yellowish brown in colour, wall smooth; pedicel hyaline.

On living leaves of *Dicliptera cuneata* Nees. Yercaud (Salem District), 27-V-1947, G. Rangaswamy.

*Pycnia* ignota; maculæ amphigenæ, orbiculares, superiori superficie fusco colore; *æcia* hypophylla aggregata, cupulata, citri colore, luteas substantias continentia; peridii cellulæ polygonæ, cum denso pariete, hyalinæ, prominenter verrucosæ,  $25 \times 18 \mu$  ( $17-29 \times 13-21 \mu$ ); *æciosporida* subglobosa, catenulata, leniter flavocolore, pariete polito vel belle verruculoso; *uredia* absunt; *telia* rara, cum æciis mixta, hypophylla, subepidermate, ut prolata pustulæ cacaotico colore apparentia; *teliosporidia* stipitata, cum duobus cellulis, clavata, apice obtuso incrassatis  $8.5 \mu$ , medio leviter constricta,  $47 \times 17 \mu$  ( $32-56 \times 12-20 \mu$ ), flavo brunneo colore, paries levis, pedicellus hyalinus.

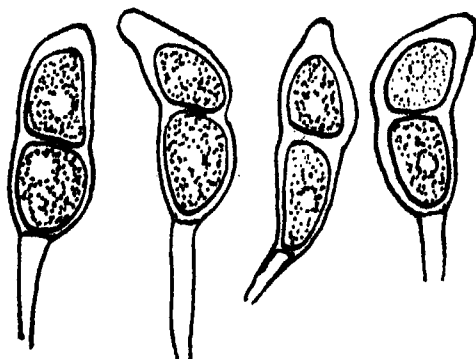


FIG. 4. *Puccinia tweediana*.—Teliospores ( $\times 400$ ).

In vivis foliis *Dicliptera cuneata* Nees. Yercaud (Salem District), 27-V-1947, G. Rangaswamy.

*P. dicliptera* Syd. has been described by H. and P. Sydow (1914) on *D. longiflora* from Formosa. This rust produces only telia, and the teliospores are smaller. In these respects it differs from the rust under study. *Aecidium tweedianum* Speg. has been recorded on *Dicliptera* sp. from India (Butler and Bisby, 1931). A specimen of this rust was obtained from the Herbarium Cryptogammæ Indiæ Orientalis, New Delhi, through the courtesy of Mr. J. F. Dastur, and compared with the rust under study. The æcial stages of the two rusts were found to be identical. In the rust under study the telia are found mixed with æcia. They remain long covered by the epidermis. Owing to the close association of the two stages it is presumed that they are of the same rust. Since the telial stage has now been found the rust is named *Puccinia tweediana*. The two stages occurring on the same host indicate the autoecious nature of this rust.

33. *Puccinia tricholanæ* (Syd.) Ramakrishnan, T. S. and K. comb. nov.

Syn. *Diorchidium tricholaenæ* Syd., *Ann. Myc.*, 1912, 10, 33.

Doidge, E. M., *Bothalia*, 1926, 2, 138.

*Uredia* amphigenous, mostly hypophyllous, subepidermal, erumpent, brown; *urediospores* oval to globose, finely echinulate, yellowish brown; wall thickened near the base, germ pores two to three,  $25 \times 22 \mu$  ( $20-40 \times 20-28 \mu$ ); *telia* amphigenous, mostly hypophyllous, crowded, more or less in lines, subepidermal, epidermis splitting open longitudinally, black; *teliospores* stipitate with long persistent hyaline flexuous pedicels up to  $108 \mu$  long; teliospores two celled, with the septum vertical, sometimes oblique, or horizontal, elliptic to oblong, chestnut brown in colour,  $38 \times 27 \mu$  ( $32-44 \times 24-32 \mu$ ).

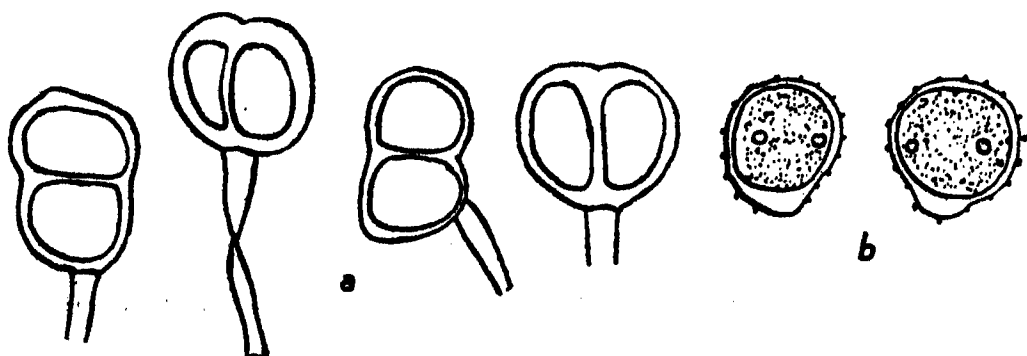


FIG. 5. *Puccinia tricholanæ*.—a. Teliospores, b. Urediospores ( $\times 400$ ).



On living leaves of *Rhynchelytrium roseum* Stapf. (*Tricholæna rosea* Nees.), Coonoor 7-VIII-1942, T. S. Ramakrishnan.

Sydow (1912) and later Doidge (1926) have recorded *Diorchidium tricholænæ* on this grass from Africa. Thurston (1940) has observed *Puccinia levis* (Sacc. et Bizz.) Magnus on this host from Brazil. *D. Wooddii* Kalch. is the type species on which the genus *Diorchidium* was founded. Sydow (1904) considered that this genus could not be maintained as different from *Puccinia* and revised *D. woodii* and several other species included in this genus transferring them to *Puccinia*. Nevertheless in 1912 he described the rust on *Tricholæna* as *D. tricholænæ*. Doidge followed the same nomenclature but mentioned that *Diorchidium* is like *Puccinia* in every respect except that the teliospores in the former have vertical septa. She also questioned the retention of *Diorchidium* as a separate genus from *Puccinia* since in many species of the latter genus a tendency for the formation of oblique or vertical walls has been noticed.

Sydow (1912) states that *D. tricholænæ* comes very near to *P. levis* but differs from it in having a higher percentage of spores with vertical or oblique septa. It may be added that *P. levis* was first described as *D. leve* and later changed to *P. levis*. Doidge described *D. tricholænæ* as having urediospores with a basal thickening of the wall and the teliospores of a chestnut brown colour. The teliospores of *P. levis* are described as brown in colour and the thickening of the urediospore wall has not been noticed. The rust under study agrees very closely with *D. tricholænæ* in all characters and is identified as such. It is considered that there is no justification for keeping this rust in the genus *Diorchidium*. In every sorus teliospores with vertical oblique or transverse septa have been observed. The presence of oblique or vertical septa has been recorded in several species of *Puccinia*. Further for this reason many species originally included under *Diorchidium* including the type species have been transferred to *Puccinia* earlier. Therefore this rust is also now revised as *P. tricholænæ*. It has not been recorded from India.

#### 34. *Puccinia baryi* (Berk et Br) Wint.

Saccardo, P. A., *Syll. Fung.*, 1888, 7, 660.

Sydow, H. and P., *Monographia Uredinearum*, 1904, 1, 737.

On living leaves of *Brachypodium sylvaticum* Beauv., Ootacamund, 10-VIII-1947, K. V. Srinivasan.

This is a new record of this rust for India.

35. *Puccinia rhynchosporæ* Syd.

Sydow, H. and P., *Ann. Mycol.*, 1913, **11**, 103.

On living leaves of *Rhynchospora* sp. Sidapur (Coorg), 17-XII-1922, K. M. Thomas.

Only the telia are present. These are hypophyllous often arranged in longitudinal series. The teliospores measure  $33-44 \times 18-22 \mu$ . The apex is rounded sometimes obtuse, thickened, or rarely almost truncated. The apical thickening ranges from 4 to  $9 \mu$ . The pedicel is hyaline and persistent up to  $60 \mu$  in length. This rust agrees with the description of *P. rhynchosporæ* Syd., the only difference being that the mesospores are rare in the present rust while they are said to be numerous in *P. rhynchosporæ*. It is different from *P. angustatioides* R. E. Stone and *P. oblongula* Jack. and Holw., two other rusts recorded on *Rhynchospora*, as the teliospores in the two latter rusts are definitely longer. It is also different from *P. consobrina* Arth. and Holw. as the telia are in rows. Hence it is identified as *Puccinia rhynchosporæ*.

36. *Puccinia coronata* Corda.

Butler, E. J., and Bisby, G. R., *Fungi of India*, 1931, 66.

Arthur, J. C., *Manual of Rusts in United States and Canada*, 1934, 152.

On the stems and leaves of *Avenastrum asperum* C. Fisch, Ootacamund, 10-IX-1947, K. V. Srinivasan.

The rust on this host agrees very closely with *P. coronata* Corda. Both uredia and telia are present. Uredia are paraphysate with capitate paraphyses. The teliospores have a crown of three to ten digitate projections. The spore size agrees with that of *P. coronata*. This is a new host for this rust.

37. *Uromyces loculiformis* Ramakrishnan, T. S. and K. sp. nov.

*Aecia* hypophyllous, solitary or in groups, in yellowish amphigenous spots, cupulate, deep seated; peridium white lacerated at the margins, becoming almost powdery, made up of polygonal, hyaline, prominently verrucose cells,  $34 \times 22 \mu$  ( $24-40 \times 16-28 \mu$ ); *aeciospores* yellowish, globose to subglobose, or polygonal, thin-walled very finely verrucose, catenulate,  $27 \times 20 \mu$  ( $20-32 \times 16-24 \mu$ ); *telia* hypophyllous, mixed with *aecia* or separate, subepidermal long covered by the epidermis, sori divided into compartments by groups of closely arranged, vertical, brown paraphyses; *teliospore* pedicellate, angular, one celled, with the apex thickened up to  $8 \mu$ , yellow to reddish brown  $34 \times 23 \mu$  ( $28-40 \times 16-28 \mu$ ); epispore smooth pedicel persistent concolorous.

On living leaves of *Chlorophytum attenuatum* Baker, Coonoor, 10-X-1947, T. S. Ramakrishnan.

Maculæ amphigenæ, flavo colores; æcia hypophylla, isolata, vel aggregata, cupulata, profunde immersa, peridii cellulæ polygonæ, cum denso pariete, hyaline, prominenter verrucosæ,  $34 \times 22 \mu$  ( $24-40 \times 16-28 \mu$ ), margine albido, lacerato; æciosporis angulato-globosis, catenulatis, flavidis,  $27 \times 20 \mu$  ( $20-32 \times 16-24 \mu$ ) pariete leviter verruculosus.

Teliis hypophyllis, subepidermalibus, multiloculatis, paraphysibus, numerosis, coalitis; teliosporidia pedicellata, polygonæ, unicellata, apice incrassatis, usque  $6-8 \mu$ , flavo vel rubrobrunneo colores,  $34 \times 23 \mu$  ( $28-40 \times 16-28 \mu$ ), episporio levi; pedicello persistente, concolores.

In vivis foliis *Chlorophyti attenuati* Baker, Coonoor, 10-X-1947, T. S. Ramakrishnan.

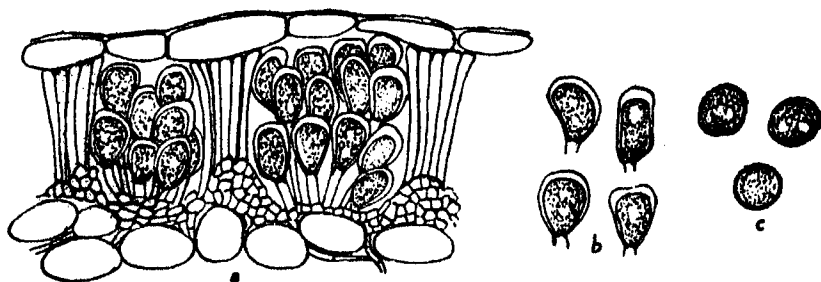


FIG. 6. *Uromyces loculiformis*.—a. Section through telia ( $\times 200$ ), b. Teliospores ( $\times 200$ ), Aeciospores ( $\times 200$ ).

This rust is found to be different from other species of *Uromyces* recorded on Liliaceous plants in the characteristic appearance of the telia with separation into compartments. The loculate telia resemble those found in some species of *Puccinia*. For this reason this rust is described as a new species and named *U. loculiformis*. It is autæcious as both telia and æcia are found on the same host.

38. *Uromyces wellingtonica* Ramakrishnan, T. S. and K. sp. nov.

*Uredia* amphigenous, but mainly hypophyllous on the lamina and sheath, minute, oval to elongate; *urediospores* pedicellate, subglobose or angled, echinulate, mikado brown to orange cinnamon,  $24 \times 22 \mu$  ( $22-28 \times 20-24 \mu$ ) with 5-6 scattered germ pores; *telia* hypophyllous, oblong to linear, black, long covered by the epidermis; *teliospores* angular, chestnut brown, apex darker coloured, thickened up to  $6 \mu$ , smooth walled,

$22 \times 18 \mu$  ( $16-28 \times 14-20 \mu$ ), pedicellate, pedicel hyaline to light brown up to  $40 \mu$  in length.

On living leaves of *Sporobolus indicus* R. Br. Wellington (Nilgiris), 9-X-1947, T. S. Ramakrishnan.

*Uredia* amphigenia exstanter hypophylla, ovalia vel elongata, minuta; *urediosporidia* stipitata, subglobose vel angularia, echinulata, brunnea vel aurantiaca cinnamomea, porum germinationis 5 vel 6; *telia* amphigenia exstanter hypophylla, oblonga vel elongata, nigra, diu epidermide tecta; *teliosporidia* polygona, castaneobrunnea,  $22 \times 18 \mu$  ( $16-28 \times 14-20 \mu$ ) ad apice incrassata, usque  $6 \mu$ , episporio levi, pedicellata, pedicelli persistenti, hyalini, vel flavo brunnei colore, usque  $40 \mu$  longi.

In vivis foliis *Sporoboli inaei* Wellington, Nilgiris, 9-X-1947, T. S. Ramakrishnan.

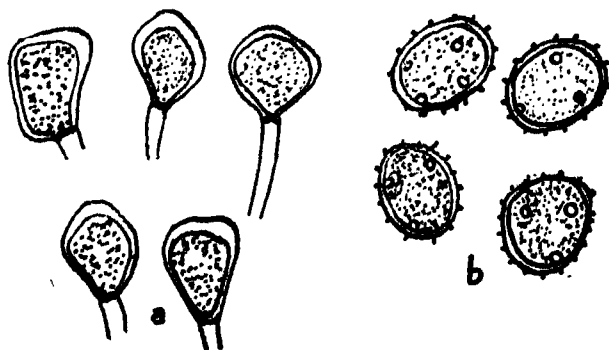


FIG. 7. *Uromyces wellingtonica*.—a. teliospores, b. urediospores ( $\times 400$ ).

On this host genus, *U. sporoboli*, *U. tenuiculis*, and *Uredo ignobilis* have been described. The rust under study is a *Uromyces* and differs from the two species above mentioned in possessing scattered germ pores in the urediospores and in the shape and size of the teliospores. It is described as a new species, *U. wellingtonica* after the place from which it was collected.

### 39. *Ravenelia ornata* Syd.

Saccardo, P. A. *Syll. Fung.*, 1912, 21, 738.

On leaves of *Abrus pulchellus* Wall. Walayar (Malabar), 15-IX-1947, T. S. Ramakrishnan and K. Ramakrishnan.

This rust was found in abundance with the uredial and telial stages. The uredia are hypophyllous, erumpent with brown, globose, finely echinulate urediospores. Paraphyses are found in plenty. These are elongated swollen at the tips and light brown in colour. Teliospores were found mixed

with the urediospores appearing as minute black dots. This rust has not been recorded from South India.

40. *Dasturella divina* Mundkur and Keshwalla.

Mundkur, B. B. and Keshwalla, K. F., *Mycologia*, 1943, 35, 201-206.

Thirumalachar, *et al.*, *Bot. Gaz.*, 1947, 108, 371-79.

On living leaves of *Randia brandisii* Gamb. Walayar, 15-IX-1947, T. S. Ramakrishnan and K. Ramakrishnan and on living leaves of *Randia candolleana* W. and A. Chittor, 22-VIII-1918, C. E. C. Fischer.

The æcial stage of this fungus has been recorded on *Randia dumetorum* Pycnia and æcia closely resembling those formed on the above host were noticed on *Randia brandisii* Gamb. Spots are found on the leaf or witches brooms are developed consisting of erect clusters of branches bearing reduced leaves. On the latter numerous orange coloured æcia are developed, hypophyllously covering the entire lower surface. Pycnia are epiphyllous appearing as black dots, hemispherical and sub-cuticular. Similar æcia have been collected on *R. candolleana* W. and A. The æcial stages of the rusts on these two hosts agree with that of *D. divina*.

41. *Cercospora kallarensis* Ramakrishnan, T. S. and K. sp. nov.

Spots amphigenous, circular or irregular, 0.2-0.5 mm. in diam., isolated or crowded, groups delimited by veins, brown; *conidiophores* hypophyllous, coremioid, densely tufted, one cluster in each spot, originating from a small stroma, unbranched, 3-6 septate,  $100-150 \times 4-6 \mu$ ; *conidia* narrowly obclavate, light olive brown, 1-3 septate, apex obtuse,  $26-60 \times 3-6 \mu$ .

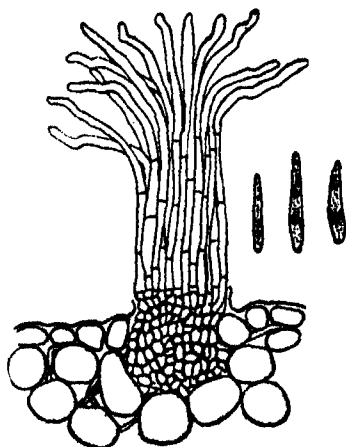


FIG. 8. *Cercospora kallarensis*.—Section of leaf showing conidiophores, conidia ( $\times 200$ ).

On living leaves of *Ficus* sp. Kallar (Coimbatore), 7-VIII-1947, T. S. Ramakrishnan and K. Ramakrishnan.

Maculae amphigenae, orbiculares, vel irregulares isolatae vel aggregate, venis delimitatae, brunneo colore, 0.2-0.5 mm. diam.; *conidiophora* hypophylla, coremioidea dense fasciculata, una quaque macula unum gerente fasciculum, quod ex parvo stromate oritur simplicia, 3-6 septata, 100-160  $\times$  4-6  $\mu$ ; *conidia* angustati obclavata, leviter olivaceo brunneo colore, 1-3 septata, apice obtuso, 26-60  $\times$  3-6  $\mu$ .

In vivis foliis *Ficus* sp. Kallar, 7-VIII-1947, T. S. Ramakrishnan et K. Ramakrishnan.

The leaves are studded with numerous spots which are clearly visible when the leaf is held against the light. When examined with a lens the coremicid conidiophores can be seen projecting as tufted growths one from the middle of each spot. Each fascicle is more or less compact and straight for nearly two-thirds of the length. Further up the conidiophores are flexuous mostly bending outwards. The spores are predominantly one septate, but conidia with two and three septa are also present. The coremioid nature of the conidiophores is peculiar. Similar conidiophores have been recorded by Solheim (1929) for *C. cercidicola* Ell. But in the fungus under study the conidiophores are simple while they are said to be branched in the other species. This fungus does not resemble any of the recorded species on this or allied hosts and is therefore described as a new species, *C. kallarensis*.

Drs. B. B. Mundkur and T. S. Sadasivan helped us with useful suggestions. Fr. A. Rapinat, S. J. of St. Joseph's College, Trichinopoly, kindly translated many of the diagnoses into Latin. The Government Lecturing and Systematic Botanist, Coimbatore, was kind enough to identify some of the host plants. Our thanks are due to them.

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### EXPLANATION OF PLATES

- Plate I. (a) Witches' broom on *Nothopegia* sp. (slightly reduced) caused by *Uræcium nothopegiae*.
- Plate II. (b) Two leaves from a witches' broom showing the sori ( $\times 1\frac{1}{2}$ ).
- (c) Section through an uræcium ( $\times 80$ ).
- (d) Section through an old spermagonium of *Guignardia nilagiriaca* showing the out growths from the wall and the multicellular filaments arising from the base ( $\times 320$ ).
- (e) An old spermagonium dissected out to show the multicellular filaments arising from the base ( $\times 320$ ).
- (f) Section through a perithecium of *Guignardia* ( $\times 320$ ).







*b*



*c*



*d*



*e*



*f*

# FURTHER STUDIES ON THE MECHANISM OF $\beta$ -AMYLASE INHIBITION BY VITAMIN C

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IN an earlier paper (Seshagiri Rao and Giri, 1942) a critical study of the mechanism of the inhibition of  $\beta$ -amylase by Vitamin C was made. The hydrolysis of Starch by amylase was found to be inhibited by Vitamin C alone and Vitamin C-Cu complex, the latter exerting greater inhibition. The oxidized products of Vitamin C by Cu oxidation were also reported to inhibit the hydrolysis of starch by the amylase. In addition to the inhibition exerted on the hydrolysis, the Vitamin C-Cu complex and the oxidation products of the Vitamin were found to inactivate the enzyme in the absence of the substrate. A correlation between the inhibition of the hydrolysis and the inactivation of the enzyme on the one hand and the oxidation of Vitamin C on the other had been established.

The observations recorded in the present paper, further elucidate the mechanism of the inhibition of the hydrolysis of starch by Vitamin C.

## EXPERIMENTAL

*Preparation of the enzyme.*—The amylase was prepared from sweet potatoes by the same method described by Seshagiri Rao and Giri (1942).

Merck's "soluble starch" was used as substrate for the determination of the activity of the enzyme.

The B. D. H. sample of ascorbic acid was used throughout this work. The vitamin was estimated by titration with iodine, after the addition of glacial acetic acid.

All the substances employed in the present investigation were of the highest grade of purity, either Merck's or Kahlbaum's products.

Water distilled twice from an all pyrexstill was used in the preparation of all reagents, substrate and Vitamin C solution.

The activity of the amylase was determined by assay of the reducing power of maltose formed in aliquots withdrawn from the reaction mixture at different time intervals according to the method of Willstätter and Schüdel (1918). The digestion mixtures contained 20 c.c. M/5 acetate buffer (pH 5.6),

20 c.c., 2% starch solution and other substances, whose influence on the activity is to be determined, the total volume being adjusted to 50 c.c. unless otherwise stated. For the determination of the activity of amylase treated with Vitamin C, oxalic acid (10 mg.) was added to the digestion mixture in order to stabilise the vitamin from oxidation. The significance of the addition of a stabilizer such as oxalic acid to the digestion mixture had been discussed in detail in an earlier paper by Seshagiri Rao and Giri (1942). Suitable blanks were always run for each experiment. The incubation temperature was  $30 \pm 0.1^\circ \text{C}$ . The activity was always expressed in milligrammes of maltose formed in the total volume of the digestion mixture.

*The inhibition of the enzymic hydrolysis of starch by Vitamin C*

The following mixtures were set up containing:—

- |   |   |             |  |
|---|---|-------------|--|
| 1 | 40 c.c. M/5 acetate + 40 c.c. starch<br>buffer (pH 5.6) | 2 per cent. |  |
| 2 | do  | do          | + 10 mg. Vitamin C.  |
| 3 | do  | do          | 10 mg. Vitamin C<br>Cu SO <sub>4</sub> , 5H <sub>2</sub> O solution containing 10.7 $\gamma$ Cu.                         |
| 4 | do  | do          | 10 mg. Vitamin C.<br>CuSO <sub>4</sub> , 5H <sub>2</sub> O solution containing 10.7 $\gamma$ Cu, oxalic<br>acid (20 mg.) |

The total volume of the reaction mixture in each case was made up to 98 c.c. with water. 2 c.c. of enzyme solution was then added and the progress of maltose production was determined. The vitamin was always added into the reaction mixture just before the enzyme was added. At stated intervals, 10 c.c. aliquots were removed for the determination of maltose and Vitamin C respectively. The results are presented in Table I.

TABLE I

*The inhibition of the enzymic hydrolysis of starch by Vitamin C*

Time of incubation in minutes	En. alone	En. + V. C.		En. + V. C. + Cu		En. + V. C. + Cu + oxalic acid	
	Activity in mg. maltose	Activity in mg. maltose	V. C. content in mg.	Activity in mg. maltose	V. C. content in mg.	Activity in mg. maltose	V. C. content in mg.
0	0	0	10	0	10	0	10
5	34	17	10	0	7.9	26	10
10	86	26	10	17	5.8	77	9.8
20	146	69	9.8	26	3.8	146	9.6
30	197	77	9.6	26	2.0	197	9.2

En.—Enzyme.

It is clear from the above results that in the case of the influence of Vitamin C alone on the hydrolysis of starch by the amylase, although the

rate of the oxidation of the Vitamin is very little and is of the same order as that of the Vitamin in presence of Cu and oxalic acid, the hydrolysis is inhibited by the vitamin.

*The influence of concentration of starch on the inhibition of its enzymic hydrolysis by Vitamin C*

With a view to find out whether the inhibition of the hydrolysis by Vitamin C alone may be due to any adsorption or complex formation of the vitamin with the substrate starch colloid, the effect of concentration of starch on the inhibition of the hydrolysis by a constant amount of Vitamin C was investigated and the results are reported in Table II.

TABLE II

*Effect of varying the concentration of Starch on the inhibition of the amyolytic hydrolysis of Starch by Vitamin C*

The digestion mixtures contained 20 c.c. M/5 acetate buffer (pH 5.6), 1 c.c. enzyme, 5 c.c. Vitamin C (5 mg.) and 20 c.c. starch solution of varying concentration. The total volume of the digestion mixture was always made up to 50 c.c. Suitable blanks were run for each concentration of starch. The mixtures were incubated at 30° C.

Concentration of starch in the total volume of the digestion mixture	Activity in mg. maltose formed in the total volume of the mixture after 30 min. hydrolysis without Vitamin C.	Activity in mg. maltose formed in the total volume of the mixture after 30 min. hydrolysis with Vitamin C.	Percentage inhibition
0.52%	115	94	18.2
0.80%	158	153	15.8
1.60%	274	248	9.4
3.20%	360	334	7.2

The results clearly show that the percentage inhibition decreases with increasing concentration of Starch, the concentration of Vitamin C being constant. Hence the inhibition of the amyolytic hydrolysis of starch by Vitamin C may be due partly to the formation of a complex between Vitamin C and the starch colloid, the modified substrate thus formed being less easily hydrolysable by the amylase.

*The influence of the concentration of the amylase on the inhibition of the hydrolysis of starch by Vitamin C*

The digestion mixture contained 20 c.c. of M/5 acetate buffer (pH 5.6), 20 c.c. starch (2%), 2 c.c. Vitamin C (5 mg.), and varying amounts of the amylase. The total volume of the digestion mixture was always made up

to 50 c.c. Suitable blanks were run for each concentration of the enzyme. The mixtures were incubated at 30° C. and the maltose was estimated after 30 min. hydrolysis

The results are presented in Table III.

TABLE III  
*Effect of enzyme concentration on the inhibition of the hydrolysis of starch by Vitamin C*

		Amount of enzyme added in c.c.	Activity in mg. maltose formed in the total digestion mixture after 30 min. hydrolysis	Percentage inhibition
Starch alone	..	1	100	18
Starch + Vitamin C	..	1	82	
Starch alone	..	2	100	9
Starch + Vitamin C	..	2	91	
Starch alone	..	4	105	6.6
Starch + Vitamin C	..	4	98	
Starch alone	..	8	105	0
Starch + Vitamin C	..	8	105	

The results indicate that the inhibition of the hydrolysis by Vitamin C decreases with increasing concentration of the enzyme. This is due to the presence of certain protective factors in the enzyme, which protect the Vitamin against oxidation (Seshagiri Rao and Giri, 1942).

The nature of the protective factor, present in the enzyme was then investigated. For this, the enzyme was inactivated by boiling it for 20 minutes and the influence of the active and the inactive enzyme on the oxidation of Vitamin C at pH 5.6 was investigated. The results are presented in Table IV.

TABLE IV  
*Oxidation of Vitamin C in presence and absence of the active enzyme and the inactive enzyme*

The following reaction mixtures were set up:—

1.	7 c. c. M/5 acetate buffer (pH 5.6)	+	2 c. c. Vitamin C (5 mg.)	+	11 c.c. water
2.	do	+	do	+	8 c.c. water
				+	5 c.c. active enzyme
3.	do	+	do	+	1 c.c. water
				+	10 c.c. active enzyme
4.	do	+	do	+	8 c.c. water
				+	5 c.c. boiled enzyme
5.	do	+	do	+	1 c.c. water
				+	10 c.c. boiled enzyme

The mixtures were incubated at 30° C. and at regular intervals of time, the Vitamin C was estimated in aliquots of the reaction mixture.

Time of incubation in minutes	mg. of Vitamin C in the reaction mixture				
	No enzyme added	5 c.c. of active enzyme	10 c.c. of active enzyme	5 c.c. of boiled enzyme	10 c.c. of boiled enzyme
0	3	5	5	5	5
15	4	5	5	4.86	4.86
30	3.2	4.8	4.8	4.8	4.8
60	2.0	4.8	4.8	4.7	4.6

The results show that the boiled enzyme also exerts almost the same amount of protection against the oxidation of the Vitamin, thereby showing that the protective factor is quite thermostable. As the enzyme used in these experiments was purified by dialysis, it can be said that the enzyme solution is accompanied by an undialysable and thermostable protective factor which protects the oxidation of Vitamin C.

It has already been observed by Seshagiri Rao and Giri (*loc. cit.*) that in addition to Vitamin C and Vitamin C-Cu complex, the oxidation products of the Vitamin obtained by oxidation with Cu also exert inhibition of the hydrolysis of starch by the amylase and inactivate the enzyme. Experiments were conducted to find out which of the oxidation products of Vitamin C was responsible for the observed inhibition. Since, in the present work the oxidation of Vitamin C by Cu was always carried out at an acid reaction (pH 5.6), the primary oxidation product will only be dehydroascorbic acid. So, the influence of dehydroascorbic acid on the amylolytic hydrolysis of starch was investigated.

*The influence of dehydroascorbic acid on the hydrolysis of starch  
by the amylase*

The dehydroascorbic acid was prepared by two entirely different methods: (a) by oxidation with Norite charcoal at pH 5.6 (Fox and Levy, 1936) and (b) by oxidation with ascorbic acid oxidase prepared from Cucumber (*Cucumis sativus*).

*Preparation of the ascorbic acid oxidase from Cucumber.*—The pressed juice was kept in the refrigerator for 24 hours and the precipitate formed was centrifuged off. The clear juice was kept in the refrigerator for another 24 hours, when a copious precipitate was formed and was again centrifuged,

The final clear juice contained all the enzyme. This was then dialysed for 16 hours in collodion bags against distilled water. The solution after dialysis was centrifuged to remove any insoluble material and the clear extract was used as the enzyme solution.

The following experimental mixtures were set up for the preparation of dehydroascorbic acid:—

*Preparation (a)*—80 c.c. M/5 acetate buffer (pH 5.6) + 15 c.c. of Vitamin C  
+ 2 gm. of pure Norite charcoal

The solution was filtered after 10 minutes, when the oxidation was complete as shown by the complete absence of any reducing power in the filtrate.

*Preparation (b)*—80 c.c. M/5 acetate buffer (pH 5.6) + 11 c.c. Vitamin C (15 mg.)  
+ 4 c.c. ascorbic acid oxidase solution.

The mixture was incubated at 30° C. for 5½ hours when all the vitamin was oxidized as shown by the complete loss of its reducing property.

The following digestion mixtures were set up containing:—

1. 20 c.c. M/5 acetate buffer + 20 c.c. starch (2%) + 1 c.c. amylase + 9 c.c. water
2. 25 c.c. of dehydroascorbic acid preparation (a) (containing dehydroascorbic acid corresponding to 5 mg. Vitamin C) do + 1 c.c. amylase + 4 c.c. water
3. 25 c.c. of dehydroascorbic acid preparation (b) (containing dehydroascorbic acid corresponding to 5 mg. Vitamin C) do do

The mixtures were incubated at 30° C. and after 30 min. hydrolysis the maltose was estimated. The results are presented in Table V.

TABLE V

*The influence of dehydroascorbic acid on the hydrolysis of starch by the amylase*

Digestion mixture	Activity in mg. maltose formed in the total volume of the digestion mixture after 30 min. hydrolysis
Amylase + Starch	100
Amylase + Starch + dehydroascorbic acid (by the Norite method)	99
Amylase + Starch + dehydroascorbic acid (by ascorbic acid oxidase method)	92

The results clearly show that dehydroascorbic acid does not affect the amyolytic hydrolysis of starch.

*The influence of dehydroascorbic acid on the amylase in the absence of the substrate*

The dehydroascorbic acid was prepared as before by (a) oxidation with norite and (b) oxidation with ascorbic acid oxidase.

The following experimental mixtures were set up containing:—

1. 20 c.c. enzyme + 12 c.c. M/5 acetate buffer (pH 5.6) + 18 c.c. water
2. do + 24 c.c. dehydroascorbic acid (containing dehydroascorbic acid prepared from 15 mg. Vitamin C by oxidation with Norite) + 6 c.c. water.
3. do + 24 c.c. dehydroascorbic acid (containing dehydroascorbic acid prepared from 15 mg. Vitamin C by oxidation with ascorbic acid oxidase) + 6 c.c. water

The mixtures were incubated at 30° C. and at known intervals of time, 2 c.c. aliquots were removed for the determination of the amylase activity. The results are summarized in Table VI.

TABLE VI

*The influence of dehydroascorbic acid on the amylase in the absence of substrate*

	Activity in mg. maltose formed in the total value of the digestion mixtures after 30 min. hydrolysis using the enzyme from experimental mixtures incubated for		
	0 min.	[30 min.	60 min.
Amylase untreated	88	88	88
Amylase + dehydroascorbic acid (prepared by norite method)	88	88	87
Amylase + dehydroascorbic acid (prepared by the ascorbic acid oxidase methods)	88	87	87

It is clear from the above results that dehydroascorbic acid does not inactivate the amylase. So, the inhibition observed in the case of the oxidation products of Vitamin C by Cu is not due to the dehydroascorbic acid formed. Thus the possible role of dehydroascorbic acid in the inhibition produced by the oxidation products of Vitamin C by Cu, is completely eliminated. Experiments were therefore conducted to see whether any cuprous oxide that may be formed by the interaction of Vitamin C with  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , was responsible for the observed inhibition.



*The influence of cuprous oxide on the hydrolysis of starch by the amylase*

**Preparation of Cuprous Oxide.**—Equal quantities of Fehlings' A and Fehlings' B solutions were mixed and the solution was brought to boiling quickly. The requisite amount of glucose was added and the boiling continued for 2 minutes. The precipitated red cuprous oxide was filtered through a sintered crucible, washed thrice with hot water and finally with alcohol followed by ether. All these operations were conducted very quickly to prevent oxidation of  $\text{Cu}_2\text{O}$ . The precipitate was then carefully dried in a closed crucible for 20 minutes in an oven at  $90^\circ\text{C}$ . The dried cuprous oxide was used in the subsequent experiments. It was always kept in a sealed tube after use.

TABLE VII

*Influence of varying concentration of cuprous oxide on the amylolytic hydrolysis of starch*

The digestion mixtures contained 20 c.c. M/5 acetate buffer (pH 5.6), 20 c.c. Starch (2%), 1 c.c. enzyme and varying concentrations of cuprous oxide ranging from 1 mg. to 10 mg. The total volume of the digestion mixture was always made up to 50 c.c. The mixtures were incubated at  $30^\circ\text{C}$ . and after 30 min. hydrolysis, the maltose was estimated.

	Amount of cuprous oxide added in mg.	Activity in mg. maltose formed in the total volume of the digestion mixture after 30 min. hydrolysis	Percentage inhibition
Amylase + Starch	..	100	0
do	1	100	0
do	2	97	3
do	5	88	12
do	10	60	40

The results clearly show that cuprous oxide inhibits the hydrolysis of starch, the inhibition increasing with increasing concentration of the cuprous oxide.

The influence of cuprous oxide in presence of Vitamin C and the effect of oxalic acid on the inhibition was next investigated. The results are presented in Table VIII.

TABLE VIII

*Effect of cuprous oxide on the amylolytic hydrolysis of starch in presence and absence of Vitamin C and Stabilizer*

The following digestion mixtures were set up:—

1. 20 c.c. M/5 acetate buffer + 20 c.c. starch (2%) + 1 c.c. enzyme  
9 c.c. water
2. do do + 1 c.c. enzyme  
9 c.c. water (containing 5 mg.  $\text{Cu}_2\text{O}$ )
3. do do + 1 c.c. enzyme  
2 c.c. Vitamin C (5 mg.)  
7 c.c. water (containing 5 mg.  $\text{Cu}_2\text{O}$ )
4. do do + 1 c.c. enzyme  
2 c.c. oxalic acid (10 mg.)  
7 c.c. water (containing 5 mg.  $\text{Cu}_2\text{O}$ )
5. do do + 2 c.c. Vitamin C (5 mg.)  
2 c.c. oxalic acid (10 mg.)  
5 c.c. water (containing 5 mg.  $\text{Cu}_2\text{O}$ )  
1 c.c. enzyme

All the digestion mixtures were incubated at  $30^\circ\text{C}$ . and the maltose was estimated after 30 min. hydrolysis.

	Activity in mg. maltose formed in the total volume of the digestion mixture after 30 min. hydrolysis	Percentage inhibition
Amylase + Starch ..	100	0
Amylase + Starch + $\text{Cu}_2\text{O}$ ..	88	12
Amylase + Starch + $\text{Cu}_2\text{O}$ + Vitamin C ..	15	85
Amylase + Starch + $\text{Cu}_2\text{O}$ + Oxalic acid ..	100	0
Amylase + Starch + $\text{Cu}_2\text{O}$ + Vitamin C + Oxalic acid ..	90	10

The results clearly indicate that the inhibition produced by cuprous oxide was enhanced by the presence of Vitamin C and that oxalic acid annuls both the inhibitions.

*Influence of cuprous oxide in presence and absence of Vitamin C and stabilizer on the amylase in the absence of the Substrate*

The following experimental mixtures were set up containing:—

1. 10 c. c. enzyme + 4 c.c. M/5 acetate buffer + 6 c.c. Water  
(pH 5.6)
2. do do do + 2 c.c. water  
+ 4 c.c. water (containing 5 mg.  $\text{Cu}_2\text{O}$ )
3. do do do + 2 c.c. Vitamin C (5 mg. 2 c.c. water + 2 c.c. water containing 5 mg.  $\text{Cu}_2\text{O}$ .)
4. do do do + 2 c.c. oxalic acid (10 mg.) 2 c.c. water + 2 c.c. water containing 5 mg.  $\text{Cu}_2\text{O}$
5. do do do + 2 c.c. Vitamin C (5mg.) 2 c.c. oxalic acid (10 mg.) + 2 c.c. water containing 5mg.  $\text{Cu}_2\text{O}$

The mixtures were incubated at 30° C. and after incubation for 30 minutes, 2 c.c. aliquots were removed for the determination of the amylase activity. The results are presented in Table IX.

TABLE IX

*Influence of cuprous oxide in presence and absence of Vitamin C and stabilizer on the amylase in the absence of the substrate*

	Time of incubation in minutes	Activity in mg. maltose formed in the total volume of the digestion mixture after 30 min. hydrolysis	Percentage inhibition
Amylase untreated ..	30	100	0
Amylase + Cu <sub>2</sub> O ..	"	89	11
Amylase + Cu <sub>2</sub> O + Vitamin C ..	"	50	50
Amylase + Cu <sub>2</sub> O + Oxalic acid ..	"	100	0
Amylase + Cu <sub>2</sub> O + Vitamin C + oxalic acid ..	"	86	14

The results show that cuprous oxide and the cuprous oxide-Vitamin C complex, both inactivate the amylase and that this inactivation is annulled to a great extent by oxalic acid.

A close study of the foregoing results indicate that the main constituent responsible for the inhibition of the hydrolysis and the inactivation of the amylase produced by the oxidation products of Vitamin C by Cu may be the cuprous oxide which is formed by the interaction of Vitamin C with Cu.

It has been previously observed (Seshagiri Rao and Giri, *loc. cit.*) that Vitamin C-Cu complex exerts more inhibition of the hydrolysis and more inactivation of the amylase than that produced by Vitamin C alone. It is well known (Hand and Greisen, 1942) that hydrogen peroxide is produced during the catalytic oxidation of Vitamin C by Cu. It is therefore thought that any hydrogen peroxide produced may be responsible for the observed increased inhibition of the hydrolysis by Vitamin C-Cu complex. So, the effect of hydrogen peroxide in presence and absence of Cu on the hydrolysis of starch was next investigated. The results are presented in Table X.

TABLE X

*The influence of hydrogen peroxide in presence and absence of Cu on the amylolytic hydrolysis of starch*

The following digestion mixtures were set up containing:—

1. 20 c.c. M/5 acetate buffer + 20 c.c. starch (2%) + 1 c.c. enzyme  
(pH 5.6) 9 c.c. water
2. do do + 1 c.c. enzyme  
0.5 c.c.  $H_2O_2$  (20 volumes)  
8.5 c.c. water
3. do do + 1 c.c. enzyme  
1 c.c.  $H_2O_2$  (20 volumes)  
8 c.c. water
4. do do + 1 c.c. enzyme  
0.5 c.c.  $H_2O_2$  (20 volumes)  
1 c.c.  $CuSO_4$   
5  $H_2O$  solution (10.7  $\gamma$  Cu)  
7.5 c.c. water
5. do do + 1 c.c. enzyme  
1 c.c.  $H_2O_2$  (20 volumes)  
1 c.c.  $CuSO_4$   
5  $H_2O$  solution (10.7  $\gamma$  Cu)  
7 c.c. water

The mixtures were incubated at 30° and the maltose was estimated after 30 minutes hydrolysis.

	Activity in mg. maltose formed in the total digestion mixture after 30 min. hydrolysis	Percentage inhibition
Amylase + starch	105	..
Amylase + starch + 0.5 c.c. $H_2O_2$	105	0
Amylase + starch + 1 c.c. $H_2O_2$	105	0
Amylase + starch + 0.5 c.c. $H_2O_2$ + Cu (10.7 $\gamma$ )	100	4.8
Amylase + starch + 1 c.c. $H_2O_2$ + Cu (10.7 $\gamma$ )	98	6.6

The results show that hydrogen peroxide alone does not inhibit the hydrolysis of starch. But hydrogen peroxide in presence of Cu exerted negligible inhibition.

#### DISCUSSION

The results reported here indicate that the inhibition of the enzymic hydrolysis of starch by Vitamin C alone, when there is very little oxidation of the Vitamin in the digestion mixture, may be due to adsorption or complex formation of the vitamin with the substrate-starch colloid, the complex thus formed being less easily hydrolysed by the amylase than the free starch. In this connection it may be of interest to refer to the work of Hale (1944) who made a similar observation during his investigations on the action

of reducing agents on hyaluronic acid and other polysaccharides, that the enzymic hydrolysis of polysaccharides like starch and hyaluronic acid after being acted upon by Vitamin C was slower than that of the unchanged polysaccharides.

Regarding the mechanism of the inhibition of the hydrolysis and the inactivation of the enzyme by the oxidation products of Vitamin C by Cu, the results reported here show that the observed inhibition of the hydrolysis and the inactivation of the enzyme may be due to the traces of cuprous oxide formed as a result of the interaction of Vitamin C with Cu, since dehydroascorbic acid and hydrogen peroxide, which form during the catalytic oxidation of Vitamin C by Cu are found to have no effect on the hydrolysis of starch.

The results reported regarding the nature of the protective factor present in the amylase, which protects Vitamin C against oxidation, indicate that the protective factor is undialysable and thermostable.

#### SUMMARY

The mechanism of the  $\beta$ -amylase inhibition by Vitamin C has been further critically examined. The observed inhibition of the hydrolysis of starch by Vitamin C alone may be due to adsorption or complex formation of the Vitamin with the substrate, the modified substrate thus formed being less easily hydrolysed than the free starch.

Dehydroascorbic acid has no effect on the hydrolysis of starch by the amylase as well as on the enzyme in the absence of the substrate.

Hydrogen peroxide alone does not inhibit the hydrolysis of starch, but in the presence of Cu it exerts a feeble inhibition of the hydrolysis.

The inhibition of the amylolytic hydrolysis of starch by the oxidized products of Vitamin C by Cu oxidation, is mainly due to the traces of cuprous oxide formed during the oxidation of the vitamin.

The protective factor present in the  $\beta$ -amylase is found to be undialysable and thermostable.

#### ACKNOWLEDGMENTS

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# LEAF DEVELOPMENT AT THE GROWING APEX AND PHYLLOTAXIS IN *HERACLEUM*<sup>1</sup>

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## I. LEAF DEVELOPMENT

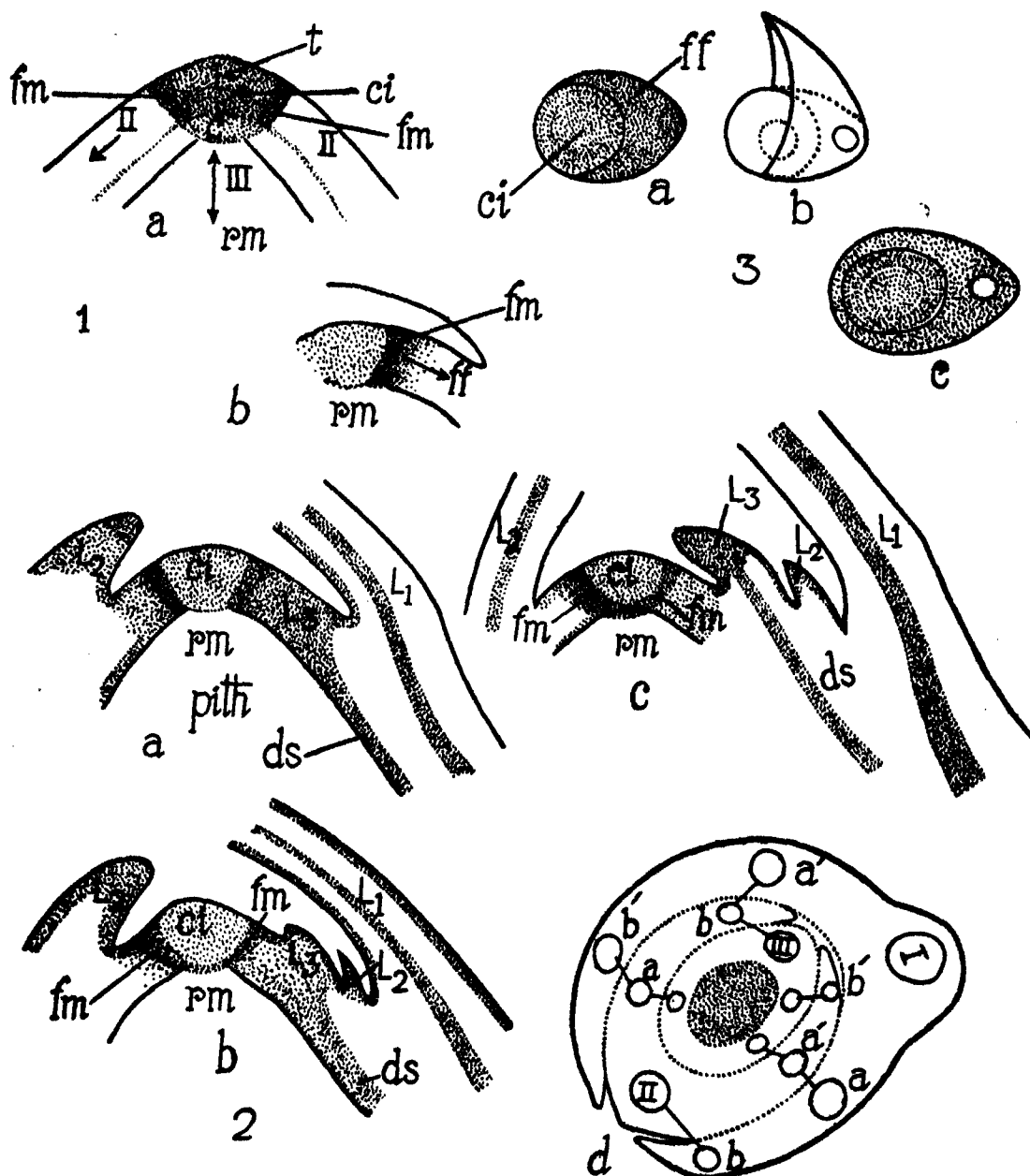
THE behaviour of the growing apex of a vegetative shoot of an Angiosperm appears primarily to lay down the leaf primordia and to maintain steady increase in dimension of the apex. The interpretation of the activity of the apical meristem is, therefore, to be expressed with reference to this behaviour.

In our study of the apical meristem at the vegetative apex of *Heracleum* (1942) we noticed the presence of at least three well defined zones. The central group of *apical initials* (I) gives rise to the *flank meristem* (II) on its sides, and the *file meristem* (III) at its base (Fig. 1 *a*). The localised activity of the flank meristem (*fm*) gives rise to the *foliar foundation* (Fig. 1 *b*, *ff*) on which takes place the foliar emergence later. The file meristem, on the other hand, is responsible for the elongation of the axis. The origin and the development of a leaf primordium is thus seen to take place in two stages, namely, (1) the *initiation*, and (2) its *emergence*.

### (1) *The Initiation:*

When a leaf is in the process of initiation the inner part of the flank meristem along the margin of the central apical initials (*ci*) at the region of the origin of a primordium becomes particularly active in cell division and stands out clearly owing to the smaller size and deeper staining capacity of the cells. This localised active growth of the flank meristem causes not only the transverse extension of the growing point on this side of the apex, but also results in its highly asymmetric growth. This was variously explained as "bulging of the histogen" (Schmidt, 1924), "active cell divisions of both tunica and peripheral layers of the corpus at the side of the growing point" (Foster, 1935) until the real import was explained by Gregoire (1935) and Louis (1935) when they named this tissue 'soubassement

<sup>1</sup> The work was completed in 1940 in the Leeds Laboratories under guidance of the late Prof. Priestley.



TEXT-FIG. 1, *a* and *b*.—Zonation of apical meristem; three zones, I, II and III; *ci*., central apical initials differentiated into upper tunica, *t*, and lower corpus, *c*; *c* occupies the apex of the inverted dome-shaped *ci*; *fm*, flank meristem, surrounds the apical dome; *rm*., rib meristem also called file meristem. Fig. 1 *b* is a part of *ci* showing localised activity of *fm* initiating leaf development; *ff*, the foliar foundation (diagrammatic).

TEXT-FIG. 2, *a*, *b*, *c* and *d*.—Initiation and emergence of primordium  $L_1$  at the growing apex. Figs. 2 *a*, *b*, *c*, show stages in the emergence of the primordium. Fig. 2 *d* is a transverse section of the shoot apex to show the origin of the median trace of  $L_1$  from the second anodic lateral, *b*, of  $L_1$ . Legends same as in Fig. 1; *ds*, desmogen strand (diagrammatic).

TEXT-FIG. 3 *a*, *b* and *c*.—Transverse sections of the shoot apex showing sectorial origin, encircling and emergence of a primordium at the growing apex (diagrammatic).

foliares' (translated *foliar buttress* by Foster, and *foliar foundation* by Majumdar) (Figs. 1 *b*; 2 *a*,  $L_3$ ).

Thus the axial component or the foliar foundation of the growth unit (*phyton*) is already laid down before the emergence of the primordium, and is expressed in the formation of the 'widest gap' on the flank of the growing apex on which the primordium (emergence) is to appear later.

(2) *The Emergence or the unfolding of the primordium:*

The emergence does not take place before the desmogen strand is well differentiated at the base of the foliar foundation (Majumdar, 1947).

When the localised activity of the flank meristem is adding to its horizontal extension, *i.e.*, to the formation of the soubassement the file meristem pushes the shoot apex upwards slowly and steadily by entering into the vacuolating extending phase (Fig. 1 *a*, *rm*).

From Fig. 2 *a* it will be seen that the so-called 'widest gap' is the direct product of the flank meristem in a particular sector of the shoot apex between the two previous primordia,  $L_1$  and  $L_2$ . Vacuolation of the adaxial surface of  $L_1$  meanwhile extends upwards into the outer few layers of the foliar foundation and in conjunction with the already vacuolated pith cells compress the innermost layers of the apical meristem and convert them into the elongated narrow desmogen cells in strands (Fig. 2 *a*, *ds*). While this is happening the apical meristem is steadily progressing upwards with the elongation of the growing point.

The desmogen strand (median trace of  $L_3$ ) is the branch of the 2nd anodic lateral of the previous primordium ( $L_2$ ) and is acropetal in its differentiation (Fig. 2 *d*). It is also continuous with the meristem of the axial component of primordium  $L_3$ . The transverse series of microtome preparations in the case of *Heracleum* shows that there is no break in the continuity of the desmogen strand from below. The cells of the strand at the upper region look more like prodesmogen cells differing from those of the flank meristem (eumeristem) in staining capacity and the mode of division.

After or simultaneously with the organization of the desmogen strand which forms the median trace in the newly formed primordium intense activity starts in the corpus derivatives of the foliar foundation just ahead of it. And as a result the cells now divide by anticlinal, periclinal and oblique walls, and soon a core of tissue is organised capped by the three layered tunica. The tunica which is characterised by its surface growth and anticlinal divisions join in its activity with the volume of tissue produced by this stimulated activity of the core of cells, and soon the smooth



surface of the flank is "heaped up" and the foliar emergence takes place (Fig. 2 *b*; Schuepp, Priestley, Foster and others). The adjustment of the inner cells takes place by symplastic growth following upon their divisions. Thus in the emergence of a primordium not only the tunica but also a few underlying layers of the meristem take part (Fig. 2 *a, b*).

With the emergence of the primordium the desmogen strand gradually differentiates upwards through the axial component of the primordium and its continuity with the apical meristem of the primordium is maintained through the transitional prodesmogen cells. The upper limit of the prodesmogen region in the primordium is marked by the progress upwards of the vacuolated marginal layers on the abaxial side of the primordium. Vacuolation on the adaxial side starts, it appears, at the end of the first plastochrone, or at the beginning of the 2nd plastochrone (Fig. 2 *c*).

The emergence starts to appear in the central region of the foliar foundation over the median strand, but the activity gradually spreads around the axis along its outer layers till it completely encircles the growing point. Thus the 'phytonic soubassement' in *Heracleum* is seen to form a complete circular segment of the axis instead of only a sector of the same as is normally the case in Dicotyledons. In this respect *Heracleum* resembles a Monocotyledon (Priestley and others, 1929, 1933, 1937; Griffith and Malins, 1930). It, however, starts as in all Dicotyledons in the form of a sector of the axis (Fig. 3 *a, b, c*).

We now see that (1) the emergence takes place in the largest available space on the conical shoot apex above the last two primordia, (2) the formation of the largest space is caused by the asymmetric growth of the growing apex, and (3) the asymmetric growth is the result of the combined localised activity of the flank and file meristems (*fm, rm*).

The question now arises, as it has occurred to many other investigators, What factor or factors determine the position of the accelerated activity at a particular region on the flank of the apex upon which the foliar emergence is to appear later under the influence of the acropetally differentiating desmogen strand. Why is it that the uniform shoot apex during the growth should result in a succession of relatively abrupt and discontinuous form changes—as raised by Priestley.

Davies (1937) classified the various causes suggested so far under *four* general heads with their exponents. Schimper and Braun (1878), Schoute (1913) and Church (1904, 1920) are among those who hold the regularity of leaf position as due to the movement or diffusion of some growth impulses, inhibitory or accelerating, in the growing region.

Schuepp (1916-17), Winkler (1901-03) and Janse (1928) think that the leaf position is determined by factors both internal and external.

But the widely accepted theory is that of Hofmeister (1868). According to him the new primordium arises in the largest gap (the angle of greatest divergence—angle of least influence of Davies) formed between its immediate predecessors, and is free from tension produced by their growth. This hypothesis has been elaborated and apparently accepted by such authors as Schwendener (1878), Van Iterson (1907), Priestley and Scott (1933), Priestley, Scott and Mattinson (1937), Snow and Snow (1931, 1933, 1935, 1937) and Davies (1937).<sup>2</sup>

The study of the shoot apex of *Heracleum* appears to bear out the correctness of this hypothesis so far as the largest gap is concerned. Near the extreme end of the growing point the maximum number of primordia that can grow together in touch with the axis has been found to be only 3, and these are in varying stages of development, from the stage of soubassement to the stage of emergence (Fig. 8).

It is but natural that the first two, particularly the second, coming before the third and occupying a large amount of space, both vertical and lateral, on the growing point should determine the region least influenced by them where active growth can take place thereby widening the surface on which the emergence of the third is to take place. The *widest gap*, therefore, corresponds with the foundation or the axial component of the primordium already laid down by the localised activity of the apical meristem.

Johnson (1926, 1931, 1933, 1936), McKay and Goodspeed (1930) and Snow and Snow (1931-37), experimentally proved that the normal position of a primordium could be altered or suppressed by the local application of growth-promoting or growth inhibitory factors, such as X-ray, apical incision and hetero-auxin.

Whether any such growth-accelerating or growth inhibitory substances are discharged by the apical initials and the last two primordia during their development,—is a question that cannot be answered with our present information. But one thing is certain that the position of the sectorial activity of the flank meristem to give rise to the soubassement is determined by the upwardly differentiating desmogen strand (median trace) which is the branch of the 2nd anodic lateral of the previous primordium. It is already known that the median trace of a primordium starts its upward course long

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<sup>2</sup> See also Sterling (1945) for a discussion on different theories with further literature,

before the formation of its axial component which, therefore, must be laid by the apical meristem just ahead of it.

Next question to be considered is in connection with the emergence of the primordium. In *Heracleum* it is seen associated with the differentiation and upward progress of its median desmogen strand at the base of the foliar foundation. It has been observed that the strand is a continuous longitudinal tract bifurcating from the 2nd anodic lateral of the previous primordium and ending in the foliar foundation itself through the prodesmogen strand (Fig. 2d). Its progress of differentiation is always acropetal through the foliar foundation to the primordium, and in the early stage its continuity with the apical meristem is always maintained through the prodesmogen cells (*cp.* Louis, 1935; Smith, 1941; Esau, 1942; Sterling, 1945, and others).

The question, however, of the first differentiation of the desmogen strand in the developing primordium has not been satisfactorily answered. Yarbrough (1934) noticed first differentiation at the base of the newly laid primordium when it was between 60–70  $\mu$  high. Priestley, Scott and Gillet (1935) noticed very early differentiation in the youngest primordium in *Alstroemeria*, whilst in the second primordium which was only 54  $\mu$  high they even found lateral strands. They also state that the first differentiation is always in continuation with vascular tissues of the axis. At another place Priestley and Scott (1937, pp. 311–12) figured (Fig. 3) and described continuity of the differentiating central strand with the apical meristem. Foster (1935) observed first differentiation at the base of the primordium in *Carya Buckleyi* var. *Arkansana*, and it is clear from his figure (Figs. 42, a, b; p. 12) that its further progress is both acropetal and basipetal. Louis (1935) described and figured the origin of procambium at the base of the primordium (isolated?) above the region of soubassement, and its further differentiation is basipetal in the latter and acropetal in the primordium. Cross (1937) found in *Morus alba* differentiation of the median trace in the corpus before the primordium has attained 75  $\mu$ .

Thiessen (1908) investigating on the seedling anatomy of *Dioon edule*, a gymnosperm, traced the acropetal differentiation from below to the apical meristem (Fig. 8, Pl. XXV; Fig. 34, Pl. XXIX). Koch (1891), Cross (1942), Crafts (1943) amongst others observed continuous acropetal development of procambium in the species of gymnosperms they studied. Gunckel and Wetmore (1946) produced evidence to show that the procambium is not only acropetal in its differentiation but also is established below the area of leaf initiation before the leaf primordium is developed (p. 294).

Esau (1942) reported continuity of desmogen strand in *Linum perenne*. Smith (1941) saw continuity in *Costus* sp., and even suggested that the position of leaf primordium is determined by the basifugally differentiating prodesmogen (?) strand. Engard (1944) showed the same thing in *Rubus* (see his Fig. 1). What Smith suggested in 1941 has been developed into a theory by Sterling (1945, 1946) while studying the origin and development of foliage leaf in *Sequoia*. He not only saw acropetal differentiation of foliar bundle prior to any sign of emergence of the related leaf primordium but connected its influence to the raising of the primordium from its buttress (p. 122, Figs. 2, 3, 4, 10; 1946, p. 381; *cp.* Gunckel and Wetmore, 1946, p. 542). Miller and Wetmore (1946) emphasized this point in the life-history of *Phlox*. They showed that at the time of the first appearance of each foliar primordium on the epicotyledonary meristem a continuous procambial strand can be traced to the new primordium from the vascular system from below (pp. 1, 9). We have noticed the same thing in *Heracleum*. This fact is perhaps in most accord with the conception of the growth unit or *phyton* developed in the Leeds Laboratory.

The extent of participation of the tunica and corpus in the emergence of a primordium appears different in different plants, and no definite pronouncement can be made in this connection. In the Angiosperms, both dicotyledons and monocotyledons, the origin of a primordium may take place exclusively from tunica, or both tunica and corpus together contribute to its formation (Schmidt, 1924; Priestley, Scott and Gillet, 1935; Rosler, 1928; Foster, 1935, 1937; Cross, 1936, 1938; Kleim, 1937; Sharman, 1938; Hsü, 1944, and others).

## II. PHYLLOTAXIS IN DEVELOPMENT

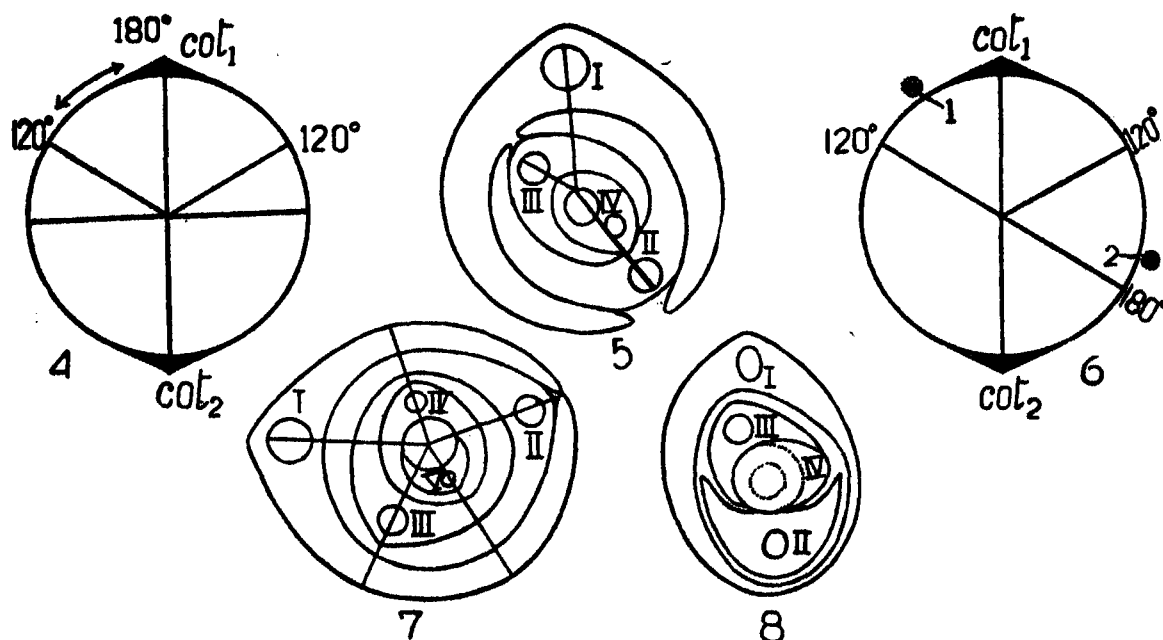
*Heracleum* has a 2/5th phyllotaxis with a clockwise arrangement of leaves. The total number of leaves produced in a year does not exceed a dozen.

Since the time of Hofmeister (1868) all workers on the anatomy of shoot apices agree that emergence of foliar primordia has a regular sequence or order, or as Church (1904) would describe it as "a rhythmic production of similar protuberances." They further agree that the different systems of phyllotaxis as seen in the adult plants are to be governed by this sequence.

From our studies in *Heracleum* we find that this order or sequence is determined not by the plastochrone or the time interval between the appearance of successive primordia but by the conditions responsible for or controlling the localised growth of the apical meristem. These conditions

cannot be regarded as hereditary or specific as variations in the arrangements of leaves are found even on the same axis. Thus in Sunflower the first pair of leaves, *i.e.*, the cotyledons, start as opposite system, but succeeding leaves pass through the decussate to pentastichous to the 3/8th system towards the apical region of the same axis. In the majority of dicotyledons the stable arrangement is reached only after the plant has grown for sometime.

In view of our studies of the growing points of *Heracleum* it appears misleading to suggest that the primordium appears in the widest gap unless of course the primordium is regarded as something apart from its foundation or the axial component. The widest gap, we have seen, is the result of the asymmetric growth due to the laying down of the foliar foundation, the initiation of which begins before the actual emergence of the primordium which happens only after the differentiation of the median strand (Fig. 2 *a, b, c*).



TEXT-FIG. 4.—Shows range of origin of the first epicotyledonary leaf on the shoot apex (diagrammatic).

TEXT-FIG. 5.—Transverse section of apical bud at the extreme end showing angular divergence between primordia I, II and III (diagrammatic).

TEXT-FIG. 6.—Shows range of origin of primordia 1 and 2 respectively at the epicotyledonary nodes, left handed spiral (diagrammatic).

TEXT-FIG. 7.—Transverse section of the apical bud showing the angular divergence between the last five primordia (diagrammatic).

TEXT-FIG. 8.—Transverse section of the apical bud showing the number of the primordia attached at a time to the axis below the free tip.

*Place of origin of a primordium: Is it definite and predetermined?*

Could we say the place of origin of each primordium is definite and predetermined? It has been made clear by researches in the Leeds Laboratory that in a higher system of phyllotaxis, say  $2/5$ th, the successive primordia cannot be separated from one another by more than  $\frac{1}{5}$  (i.e.,  $180^\circ$  angular divergence), or can be closer than  $\frac{1}{5}$  ( $120^\circ$  angular divergence) of the circumference. The range of origin thus given is  $\frac{1}{5}$  of the circumference and the initiation of the primordium can start at any point within this range (Fig. 4). Whether the initiation should be nearer  $120^\circ$  or  $180^\circ$  depends not only upon the nature and extent of the growth of the last two primordia but also upon the direction of the desmogen strand, the future median trace, differentiating from below upwards. The position of a new primordium can be described, therefore, definite and predetermined so far as the range of its origin is concerned, but not with regard to its point of origin which to the present writer appears to be determined by the desmogen strand, its median trace, coming from below.

*Variation in the angular divergence at the earlier stages of primordial differentiation*

In *Heracleum* which has a  $2/5$ th phyllotaxy, normal angular divergence should be  $144^\circ$ , but as a matter of fact it is seen to vary in different apices from  $144^\circ$  to  $160^\circ$  between the successive primordia which are in different degrees of development, the widest divergence being always between the youngest primordia near the tip (Fig. 5).

It is no doubt difficult to determine the angular divergence with any degree of exactitude in *Heracleum*. Due to the highly asymmetric growth of the shoot apex a perfect transverse section is hardly possible and then to fix the 'relative point which represent the centre of the symmetry of the axis' (Priestley, 1937, p. 384) is not a simple affair. The angular divergences given here are rather approximate yet the variations noticed are both apparent and natural.

It is still a matter of controversy if the arrangement of leaves, hence angular divergence, in the bud and on adult stems is the same. Salisbury (1931) believed that lateral displacement due to mechanical or other causes brings about the variety of divergences present in the species or even in the same individual (p. 542). Priestley (1937) thinks that the relative position of a particular leaf on the axis may not remain exactly the same throughout its development (p. 389). Davies, however, found no difference in the two regions in *Ailanthus*.

The variations in the angular divergence seems to the present writer a natural sequence in the seedlings of *Heracleum*. The plant has a very small number of leaves to reckon with. The two cotyledons are epigeous and grow for sometime. The tip at this stage is far too rudimentary, and so there always is a limit to spatial adjustment in the early stages. Thus there is hardly any room free from the influence of cotyledons, 1 and 2, for a large number of primordia to be laid down. The region near about  $120^\circ$  appears to be free from the influence of the two cotyledons which are still growing and expanding, and the first primordium is laid down quite near the  $120^\circ$  limit. But the second primordium has got more free space for its origin and its position is shifted towards  $180^\circ$  (Fig. 6). Similarly with the growth in volume of the apex and with more food at their disposal the points of origin are shifted more and more towards the normal angular divergence of 2/5th phyllotaxis, i.e.,  $144^\circ$ .

There is another contributory factor towards the cause of variation in the angular divergence at the earlier stages. A primordium starts as a sector of the axis and then it spreads superficially around the expanding inner primordium. The cross section of the axis which is made up of the confluent axial components of the primordia is, therefore, never circular in outline but always periodically highly asymmetrical with reference to the organic centre of the axis. Moreover, the axial components are in different stages of growth and development, particularly with reference to their anodic and cathodic sides (Fig. 7). The equilibrium is reached only at a later period in their development (see below).

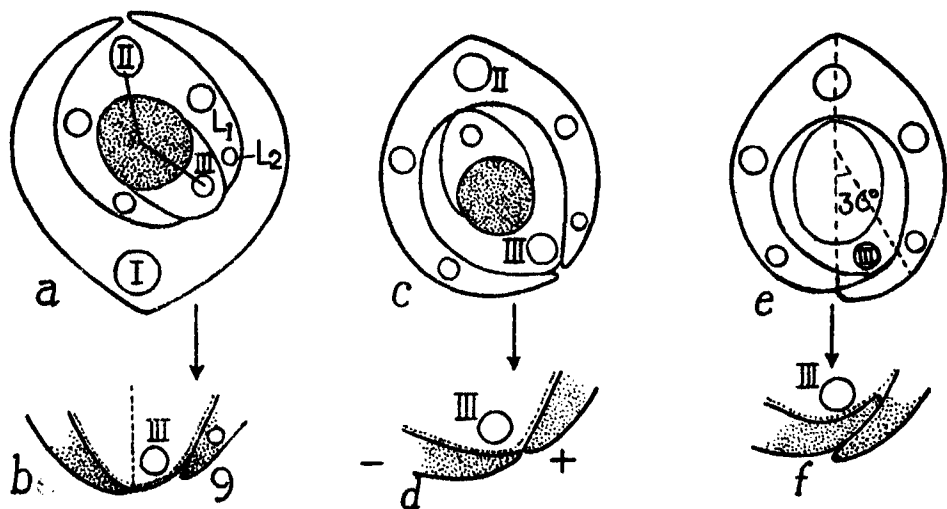
*Non-correspondence of the position of the abaxial fold (ridge) with that of the median strand and the unequal growth of the two wings of a primordium*

Each primordium of *Heracleum* starts as a sector of the axis and then spreads laterally till it completely encircles the inner primordium which has already started differentiation.

At the beginning the foliar foundation is convex on the abaxial side (Fig. 9 a), and free from the embrace of the outer primordium. The two sides, anodic and cathodic, of pr. 2 now begins to extend laterally and grow equally vigorously for sometime. The progress of the anodic side, however, slows down with the differentiation of its second anodic lateral ( $l_2$ ) from which the median strand of pr. 3 is directly derived (Fig. 9, a, b). The slowing down of the growth may be due to the diversion, at least for the time being, of the food supply to pr. 3 which is now fast developing.

On the cathodic side of pr. 2 the second lateral strand is from the median strand of pr. 1 (Fig. 9 a), and apparently its marginal meristem gets a steady

supply of food material to push its growth further along the dorsal surface of pr. 3 till it meets the anodic margin. As a consequence a ridge is now formed above the median trace where the two sides meet (Fig. 9 c, d). Though the growth of the cathodic side now stops that of the anodic side is resumed or accelerated and the margin soon overlaps the other by about 1/10th of the circumference,  $36^\circ$  (Fig. 9 e, f).



TEXT-FIG. 9 a-f.—Transverse sections of the apical bud showing non-correspondence of the abaxial ridge and the median trace in the early stage of primordial differentiation (diagrammatic).

In a 2/5th system where the leaf base completely encircles the axis as in *Heracleum*, the growth of the cathodic side over that of the anodic side by about 1/5th of the circumference seems inevitable, and in so doing it causes the formation of the ridge over the median strand which is placed near the end of the anodic side. This fact also shows that the final adjustment of the primordium on the axis is a late phenomenon.

#### *Clockwise and Counterclockwise arrangement*

The arrangement of leaves in *Heracleum* is clockwise. Whether the spiral should be clockwise or anticlockwise in a higher system of phyllotaxis does not appear to have a specific significance. As long ago as in 1894 Weisse pointed out that leaf arrangement on stem depends on the manner in which the system originates. Cook (1914) stated that it is not a specific character because equal ratios of left handed and right handed spiral occur in *Pinus austriaca* and *P. pumilio*. The same thing was observed by Imai (1927) in the Japanese Morning Glory. Lugard (1931) found variations in the branches of the same cotton plant. Priestley and Scott



(1933) think that the leaf arrangement is determined by the position of the first primordium. Snow and Snow (1935) showed by incision experiment that leaf arrangement depends on the arc covered by the first few leaves formed at the apex and the shape and position of the members below them with which they make contact. Davies and Theiss (1937) believed that normal symmetry depends upon a balanced distribution of the leaves, but in another paper Davies (1937) thinks that in *Ailanthus* the fourth primordium (including the two cataphylls) is the controlling influence in determining subsequent arrangement in a clockwise or counterclockwise direction.

The study of seedling apex of *Heracleum* shows that the anti-clockwise or clockwise direction of the spiral depends on the position of the first primordium with reference to the position of cotyledon 2 (Fig. 6). The primordium has equal chance of being laid down on the right or left of it because the growth is about the same on both sides. If it is laid down on the right of it the spiral will be right handed, and if on the left, as in *Heracleum* it is left handed.

Allard (1946) did not find a single instance in Potato of the reversal of the spiral from that which began above the cotyledons on the main stem. He says that the direction of the spiral never changes once it has started above the cotyledons. He, however, found occurrence of clockwise and counterclockwise spirality in the phyllotaxis of Tobacco at about 50:50 proportion, which shows origin of the first primordium to be a chance origin. How far this chance is determined by the acropetally differentiating median trace from below has not been definitely worked out though Miller and Wetmore (1946) saw it in *Phlox* (see p. 4 a). The 50:50 ratio has also been reported by Macloskie (1895) in *Spiranthes præcox*, by Koriba (1914) in *S. australis*, De Vries (1909-10) in *Dipsacus sylvestris*, Ikeno (1923) in *Plantago major* var. *asiatica*, Davies (1937) in *Ailanthus altissima*, Cook (1914) in *Pinus austriaca*, *P. pumilio*, Lugard (1931) in Egyptian Cottons, Imai (1927) in Japanese Morning Glory, Sweet Potato, etc. These authors also found that these arrangements of leaves on the stem are not specific and heritable.

#### *Number of primordia on the growing apex*

On the growing points studied in serial microtome sections, each 8  $\mu$  in thickness, only one shows three primordia attached to the axis at the same level. Two primordia, numbers 3, 4 (Fig. 8) are in the meristematic condition and in the initial stage of foliar foundation. The other primordium (pr. 2) has just started vacuolating at the abaxial surface and is at the point of separation from the axis,

The two foliar foundations, 3 and 4, are in different stages of growth and elongation along the axis. The 4th or the youngest has grown  $72\mu$  and the 3rd,  $104\mu$ , the upper end of both being at the same level,  $16\mu$  below the growing point of the apex. At this stage the second primordium has grown  $144\mu$  along the axis and has separated from it at a level  $32\mu$  below the growing tip, while the first primordium has grown  $152\mu$  along the axis and has separated from it at a level  $104\mu$  below the growing point. The comparative growth of the primordia stands thus:

Primordium	Growth along with the axis	Free growth	Total growth
1	$152\mu$	$776\mu$	$928\mu$
2	$144\mu$	$192\mu$	$336\mu$
3	$104\mu$	$0\mu$	$104\mu$
4	$72\mu$	$0\mu$	$72\mu$

Axis free above the level of Pr. 3 and 4— $16\mu$

The foliar foundation of *Heracleum* like that in Monocotyledons has a ring-like insertion at the adult stage, but when it is just initiated it occupies comparatively a small sector of the axis. Gradually it extends radially, tangentially and vertically along with the elongation and expansion of the shoot apex. This ring-like growth as Priestley (1938) has pointed out 'necessitates the insertion of successive primordia one above the other and also inside one another' (p. 172). This is exactly the picture we find at the vegetative apex of *Heracleum*.

At level  $32\mu$  below the growing point three primordia, 2, 3 and 4, are seen attached to the axis: number 2, the oldest, only through a small portion and on the point of separation from the axis, foliar foundation 3 occupying about half, and 4 about a third of the circumference of it. At this level there is no room for the origin of the next foliar foundation 5, at least there is no visible sign of it.

If we consider the tremendous growth of primordia 1 and 2, the reason for the slow growth and differentiation of pr. 3 and 4 and the apical meristem becomes apparent. The first two primordia during their intensive growth must have drawn and be drawing a huge amount of formative material leaving apparently only a small amount for the axis and the two axial components, pr. 3 and 4, to draw and feed upon for their growth and adjustment. It is thus only reasonable to expect that the plastochrones in the case of *Heracleum* are comparatively a little longer. Miss Smith (1941) noticed

only one meristematic primordium at the growing apex of *Costus* and thought the plastochrone to be disproportionately long in comparison with the time taken for a leaf development at the apex. She thinks this is due to the existence of an interphase during which the preceding primordium enters into the vacuolating phase of its growth. This interphase, she suggests, corresponds with the resting period observed by Schuepp in *Artocarpus* (9:20). In *Heracleum* there is no resting period during the short season of its annual manifestation of life. The number of leaves produced is comparatively small, therefore, the comparatively long plastochrone appears to be the result of a period of slow growth during which the two preceding primordia and the apical meristem grow and adjust themselves.

The presence of three primordia at the level  $32\mu$  down the growing point shows that the 'limiting extreme values of the angular divergences' in the present system of phyllotaxis are to be found between  $\frac{1}{2}$  and  $\frac{1}{3}$  systems, a point made clear perhaps for the first time in the Leeds Laboratory.

The present writer does not know if the fact of the occurrence of only three primordia at the same level near the growing tip has any bearing on the view first held by Haberlandt (1914, p. 714, note 40), and later supported by Salisbury (1931, p. 542) that the apical meristem of the Angiosperms is a multicellular equivalent of the three-sided apical cell of primitive plants. This seems highly speculative in the absence of authentic data.

#### SUMMARY

The origin of leaf at the growing apex and phyllotaxis in development have been studied in *Heracleum*. The leaf originates in two stages, namely, (1) the initiation, and (2) the emergence.

The *initiation* takes place by the localised activity of the flank meristem on one side of the apex resulting in the formation of the soubessement (Gregoire), or the foliar foundation, *i.e.*, the axial component of the primordium.

The *emergence* takes place only after the desmogen strand, the future median trace of the emergence (primordium) has differentiated up to the base of the foliar foundation. Intense activity starts in the corpus derivatives of the foliar foundation just ahead of the desmogen strand resulting in the organization of a core of meristematic tissue capped by three layers of tunica. Soon the smooth surface of the foliar foundation is "heaped up" and the foliar emergence has taken place followed closely by the desmogen strand as its median trace in the process of differentiation.

A leaf is thus composed of two parts, namely, the axial component and the free limb, and these together make up the *phyton*, or the growth unit.

*Heracleum* has a 2/5th phyllotaxis with a clockwise arrangement of the leaves. The angular divergence is  $144^\circ$ . Developmental studies show that this is true only on adult shoots. At the growing point the angular divergence is seen to vary between  $144^\circ$  and  $160^\circ$ , the widest divergence being noticed between the youngest primordia near the tip. The reasons of these variations have been discussed.

Determination of the cause or causes responsible for the orderly appearance of primordia at the shoot apex has been so far a speculation. Developmental studies offer a solution. Place of origin of a primordium is primarily determined by the acropetally differentiating desmogen strand which separating from a lateral strand of a primordium down the axis follows a definite course upwards and enter the new primordium as its median trace. Its origin and development precedes that of the primordium. The widest gap is really provided by the formation of the foliar foundation.

Non-correspondence of the position of the abaxial fold or ridge with that of the median strand, and the unequal growth of the two sides of a primordium in the early stages of development have been discussed.

Other points of interest in this connection, such as, number of primordia in contact with the axis at a time at the growing point, clockwise or anti-clockwise disposition of the genetic spiral have also been discussed in this paper.

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# STUDIES ON THE *DINODERUS* BORER IN BAMBOO

## Part I. A Note on Copulation in *Dinoderus* sp.

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### ABSTRACT

It is shown that copulation in *Dinoderus* sp. takes place definitely outside its tunnels in bamboos, soon after emergence. A description of the egg is also included.

### 1. INTRODUCTION

IN spite of its great economic importance, very little attention has been paid to the study of the biology of *Dinoderus* sp. (Bamboo-borer) in India, and whatever observations have been published on the subject so far seem to be based mostly on imperfect evidence.

Beeson's account<sup>1</sup> of the life history of *Dinoderus* sp. leaves many important points uncertain. Regarding copulation in *Dinoderus* sp., he has stated that the beetles, after emergence, go to a different or the same bamboo joint and tunnel inside for mating and oviposition. The possibility of *Dinoderus* adults mating inside appeared to be rather remote and a systematic investigation of the problem was undertaken. The results of the investigations which are recorded in the present paper have conclusively shown that mating takes place outside, soon after the emergence of the beetles from the bamboo.

### 2. COPULATION IN *DINODERUS* SP.\*

(i) *Observations on beetles taken out of bamboos.*—A number of immature adults taken out of bamboo tunnels from different regions was dissected and a thorough study of the reproductive organs of both the sexes was made. A systematic examination of the testes showed spermatozoa in all stages of development, the motile stage being found in the males about to emerge. The ovaries also showed various stages of development.

\* Note added in proof: Since the writing of the paper, the species has been identified to be *Dinoderus ocellaris* Steph.

(ii) *Observations on 'Emergent' beetles.*—The first batch of adults emerged in the third week of February 1947 from infested bamboos. Two of these were found to be females on dissection. The spermathecae were removed in sodium chloride solution (0.4%), teased and examined under high power magnification. Both the preparations revealed innumerable motile spermatozoa stored in the pouches. Dissections of the emerged male adults also revealed active spermatozoa near the junction of the testes with the vasa deferentia.

On the second occasion several fresh females collected 12–18 hours after emergence were dissected, and many of these also showed the presence of spermatozoa. The spermathecae of females actually taken out of the tunnels in bamboos, however, failed to show the presence of spermatozoa, indicating that mating had not taken place inside bamboo before emergence.

It is thus evident that mating usually takes place after the emergence of the beetles (within 18 hours).

Actual mating was also observed in newly emerged adults when these were released in a broad glass dish. The male approached the female from behind, mounted on her back and held her in position by means of his fore-legs. The process of copulation was of very short duration, being completed in about 30 seconds. This might perhaps account for its not having been noticed earlier.

Some important questions arising out of the above findings, namely, whether the males accompany the females when fresh bamboos are attacked, if so, what is the nature of the role they play, and whether one mating is sufficient for the fertilisation of all the eggs laid by a female, are under investigation.

### 3. EGG OF *DINODERUS* SP.

In bamboos, the eggs were found in the vessels of the soft fibrovascular bundles, closely entrenched in the grooves. Owing to their extremely

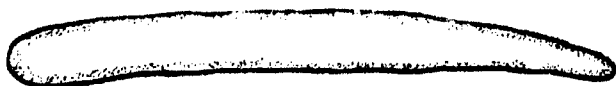


FIG. Egg of *Dinoderus* sp.  $\times 80$

fragile nature, the eggs could not be isolated from bamboos for detailed study. When caged with crushed grains of sweet maize,<sup>2</sup> *Dinoderus* adults were found to feed normally and eggs were laid loosely in the frass produced by feeding. The eggs so obtained differed very slightly in shape and appearance from those laid in bamboos.

The egg was elongate, elliptical in shape, with one end slightly narrower and more pointed than the other. The freshly deposited eggs were opalescent white, gradually changing to a dull white with age. The surface was perfectly smooth in appearance at first, but contracted a little before hatching. The length on the average was 1.2 mm. and the width 0.11 mm. throughout, excepting at the tips.

When the eggs were incubated at 30° C./75% R.H, normal hatching took place, the incubation period being 5-6 days. The newly hatched larva was almost cylindrical in shape, without the greatly enlarged thorax, so characteristic of the Bostrychid larva.

The exact number of larval instars and the duration of each stage are being studied under laboratory conditions.

#### 4. ACKNOWLEDGEMENT

The authors' thanks are due to the Deputy Chief of the General Staff (Weapons and Equipment) and the Director of Technical Development, Army Headquarters, New Delhi, for kind permission to publish the paper.

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# STUDIES ON THE *DINODERUS* BORER IN BAMBOO

## Part II. Identification of Sexes in *Dinoderus* sp.

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(Communicated by Dr. T. S. Subramanian, F.A.Sc.)

### ABSTRACT

The sex differentiating structures in the pupal stage of *Dinoderus* sp. are described. A technique for rearing the larvæ under artificial conditions is also included.

### 1. INTRODUCTION

THE identification of sexes in living adults of *Dinoderus* sp. would appear to have been attempted by a number of workers but no success has so far been reported in this matter. The importance of the problem, however, need hardly be stressed, especially in connection with the study of the biology of the insect.

The present authors have examined live adults\* for secondary sexual characters, but could not find any constant and reliable structural differences. The pupæ were then studied and these were found to show certain well-defined and constant male and female characters.

To make use of the identification of sexes in the pupal stage for practical purposes, it was necessary to ensure that the pupæ thus sorted out could be successfully developed into adults under artificial conditions.

In extracting the pupæ from their natural habitat, a large percentage was injured in the process, and further, fine frass found deposited on the tips of their abdomen (wherein lie the distinguishing structures) made the sex determination difficult. To overcome these difficulties, a technique was evolved by which the last stage larvæ, removed from the bamboos, could be reared under artificial conditions and normal pupæ obtained therefrom.

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\* Note added in proof: Since the writing of the paper the insects used in the investigation have been identified to be *Dinoderus ocellaris* Steph.

## 2. TECHNIQUE FOR DEVELOPING PUPAE UNDER ARTIFICIAL CONDITIONS

Larvæ which were deemed to be in the last stage were collected from the bamboos in petri dishes and then reared in glass tubings in the following manner.

One end of the tubing (75 mm.  $\times$  5 mm.) was plugged with cotton-wool and the larval food (Bamboo dust + 5% each of starch and glucose on the weight of the bamboo dust) was filled in the tube to about 12 mm. length. One larva was then introduced into the tube and the food put over it to another 12 mm. length. Another grub was then introduced and in this way four larvæ were packed in one tube, alternating with the food material. The other end of the tube was then plugged with cotton-wool.

The tubes were maintained at about 30° C. and 75% R.H. The larvæ were usually visible through the glass and pupation could be observed. As and when the pupæ were formed, they were taken out and the remaining larvæ put back.

## 3. SEX DIFFERENTIATING STRUCTURES IN THE PUPAE

The pupæ were examined under a binocular microscope ( $\times$  30).

Fig. 1 shows the structures as seen from a ventral view of the 9th abdominal segment in male and female pupæ.

Fig. 2 shows the lateral view of the same structures.

In the male pupa, there is seen a pair of papilla like appendages, having slight depressions on either side, their distal ends being narrower than the proximal ends. They are almost touching each other at the bases and tips.

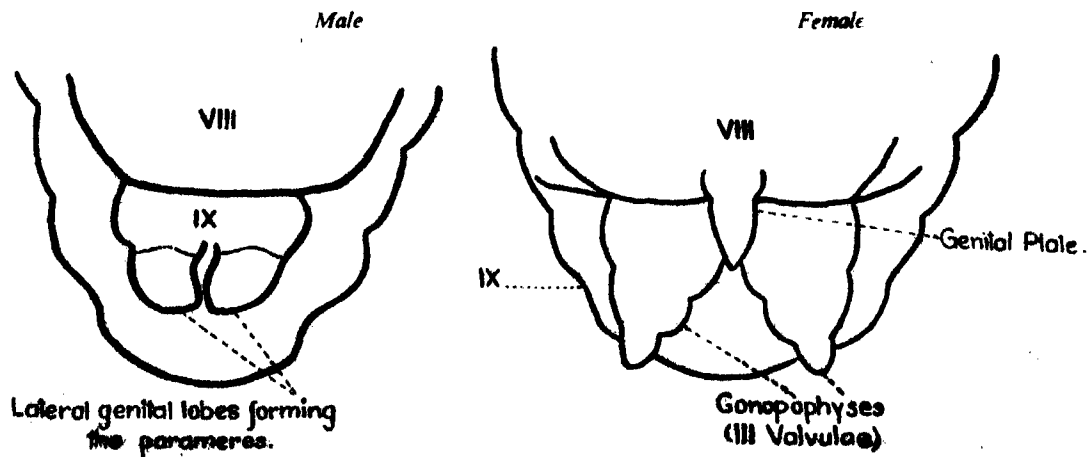


FIG. 1. Last Abdominal Segments of Pupa (Ventral View)

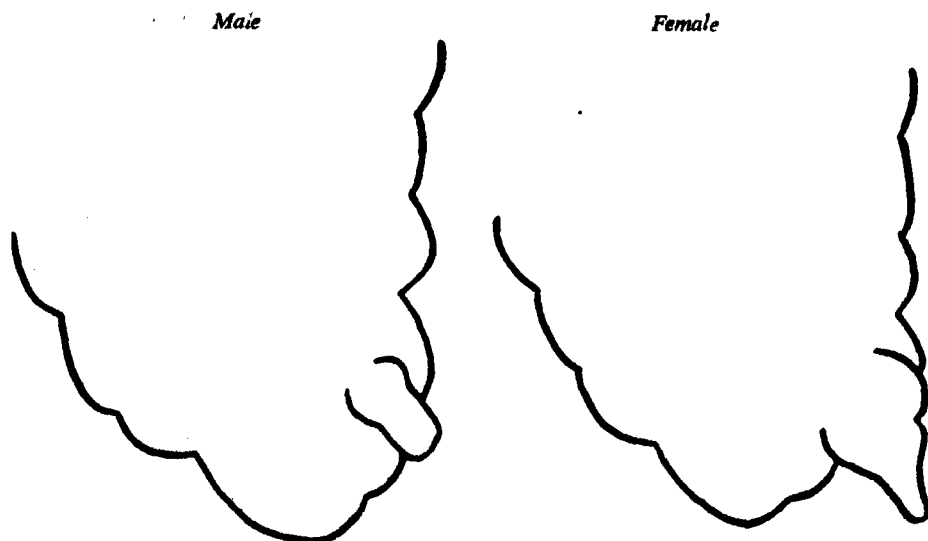


FIG. 2. Last Abdominal Segments of Pupa (Side View)

These appendages may be termed the 'lateral lobes of the genital papillæ'. They arise from the posterior margin of the 9th sternum and form the parameres of the male. Behind and partly overhanging the appendages there is a lobe which seems to be incompletely divided. This is the modified 9th sternum. The median lobe of the genital papillæ forming the 'ædeagus' of the adult, is situated below the 'laterals' and is not distinctly visible.

In the female pupa, there is seen a pair of much larger conical appendages, originating from the same position as those of the male. The bases of these appendages lie close together, but distally the structures diverge with the result that their tips are wide apart. They represent the coxites of the 9th sternum and may be termed the 'gonapophyses' or the '3rd valvulæ'. The median structure in between the gonapophyses is the genital plate.

The appendages when viewed ventrally are fully visible in the female and would seem to extend beyond the margin of the last abdominal segment, while in the male they have the appearance of a pair of beads, which are too short to reach the posterior margin of the abdomen.

The margins of the last abdominal segment in both the sexes are almost similar, with the only difference that in the male there are slight projections on the sides of the segment.

These structures can be most easily differentiated when the pupæ are put partly on their sides. Artificial light and a dark background are helpful.

#### 4. DEVELOPMENT OF PUPAE INTO ADULTS

The male and female pupæ thus differentiated were kept in Petri dishes on a little quantity of bamboo dust. The dishes were stored in desiccators maintaining 75% R.H. and incubated at approximately 30° C.

Almost hundred per cent. healthy adults have been successfully reared out of such pupæ in the course of four to five days.

The best way to handle the pupæ is to collect them by means of camel-hair brush on a piece of paper held close to the pupæ.

#### 5. SUMMARY

The paper primarily deals with the sex differentiating structures in the pupal stage of *Dinoderus* sp.

The differentiating structures lie on the ventral side of the 9th abdominal segment.

A technique for rearing the larvæ in glass tubings under artificial conditions till they turn into pupæ has also been described.

Pupæ handled for identification of sexes have been successfully developed into normal adults for experimental purposes.

#### 6. ACKNOWLEDGEMENT

The authors' thanks are due to the Deputy Chief of the General Staff (Weapons and Equipment) and the Director of Technical Development, Army Headquarters, New Delhi, for kind permission to publish this paper.



# **STUDIES IN SAMPLING TECHNIQUE**

## **I. Estimation of Whitefly (*Aleurolobus Barodensis*) Incidence in Sugarcane**

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Received August 26, 1946

### **I. INTRODUCTION**

THE determination of the adequate sampling size in the efficient estimation of whitefly incidence is of much practical importance. The solution of the problem lies in being able to adopt the appropriate method of sampling and to determine its magnitude for estimation of the incidence with any desired accuracy in an infested plot of given area. The common practice of estimating the incidence of whitefly is in terms of the number of puparia per square inch of the leaf area. The process of calculating the incidence, therefore, involves the enumeration of the total puparia per leaf and the calculation of the leaf area. If this enumeration is required to be completed even in one clump in its entirety, the labour involved becomes obviously too huge. Thus the issue that arises is to investigate whether fewer canes in a clump, fewer leaves in a cane and further if partial examination of a leaf would serve the purpose of estimation.

The incidence of whitefly has been found to be highly variable even within a comparatively small area infested with it and it is thought that the sampling requirements might differ from plot to plot varying according to the intensity of incidence.

The aim of the present note is to answer with practicable accuracy the question of sampling as enunciated above. An attempt will be made to throw light on the nature and size of samples varying if at all with low, medium and severe infestation.

Three broad divisions have been made of the type of infestation to enable the survey staff easily recognise them under field conditions so that the right type of sampling procedure may forthwith be applied.

### **II. MATERIAL**

The material for the present study consists of the complete enumeration of whitefly incidence in two plots of about 1/40th acre in size (60' × 18'). The rows have been divided each into units of 3 ft. to facilitate the sampling

TABLE  
Number of clumps, canes and leaves (affected)

Row 1										Row 2										Row 3									
Units	Clumps	Canes	Total			Affected				Clumps	Canes	Total			Affected				Clumps	Canes	Total			Affected				Clumps	Canes
			G.L.	D.L.	T.L.	G.L.	D.L.	T.L.				G.L.	D.L.	T.L.	G.L.	D.L.	T.L.				G.L.	D.L.	T.L.	G.L.	D.L.	T.L.			
1	3	9	89	36	125	78	7	83	2	12	95	96	191	79	27	106	1	3	28	10	38	21	6	27					
2	2	6	70	47	117	57	12	69	2	6	75	36	111	64	16	80	2	10	110	50	160	98	37	135					
3	2	7	88	63	151	75	16	91	2	6	71	51	122	60	28	88	3	7	95	43	99	50	28	78					
4	1	6	56	57	113	48	16	64	2	7	77	48	125	55	18	73	3	12	116	69	185	85	56	141					
5	2	7	80	56	136	64	13	77	2	11	17	74	91	64	20	84	2	6	72	33	105	50	25	75					
6	1	10	106	91	197	71	29	100	1	4	53	25	78	37	12	49	3	9	114	38	152	78	32	110					
7	2	8	90	74	164	77	19	96	1	3	40	23	63	27	16	43	2	5	52	22	74	42	19	61					
8	1	5	57	37	94	40	19	59	1	4	58	27	85	29	19	48	1	2	22	6	28	15	6	21					
9	3	10	102	69	171	71	36	107	2	9	100	67	167	38	41	79	2	8	90	32	122	63	21	84					
10	1	9	97	83	180	42	25	67	2	7	76	46	122	37	26	63	1	2	31	10	41	25	6	31					
11	2	8	91	66	157	37	22	59	1	6	49	29	78	42	20	62	3	12	132	41	173	81	30	111					
12	1	6	73	45	118	36	13	49	2	9	101	76	177	79	47	126	1	5	40	19	59	24	13	37					
13	3	7	83	37	120	30	21	51	2	8	86	51	137	46	31	77	1	4	32	12	44	26	9	35					
14	3	11	122	81	203	54	42	96	2	13	167	72	239	96	45	141	2	10	97	32	129	52	26	78					
15	2	7	85	52	137	44	13	57	1	9	87	27	114	55	14	69	2	7	80	29	109	81	18	49					
16	2	6	68	29	97	38	15	53	...	...	...	...	...	...	...	...	1	3	35	14	49	15	9	24					
17	1	5	69	36	105	33	10	43	2	8	92	32	124	65	25	90	1	5	47	18	65	27	9	36					
18	1	3	34	20	54	11	4	15	2	8	77	36	113	41	26	67	1	5	52	18	70	22	10	32					
19	2	9	119	58	177	44	13	57	2	7	78	43	121	48	36	84	1	13	131	46	177	34	26	60					
20	2	8	103	77	180	35	16	51	1	4	51	22	73	38	17	55	2	9	83	34	117	43	12	55					
21	3	9	104	54	158	37	16	53	2	9	101	44	145	63	30	93	2	5	52	17	69	28	9	32					
22	2	5	56	42	98	22	11	33	1	6	71	27	98	56	19	75	2	6	82	25	107	30	17	47					
Total	42	161	1842	1210	3052	1042	388	1430	35	156	1622	952	2574	1119	533	1652	39	148	1554	618	2192	935	424	1359					

% age affected Green leaves

on the basis of Green leaves 56.57

68.99

60.17

% age affected Green leaves

on the basis of total leaves 34.14

43.47

42.66

% age affected leaves on the

basis of total leaves 46.85

64.18

62.00

Note. G. L. — Green leaves. D. L. — Dry leaves. T. L. — Total leaves.

For the whole plot % age affected

For the whole plot % age affected

green leaves = 58.51.

leaves = 49.43.

I

and otherwise) that existed in the plot—Plot (1)

Row 4								Row 5								Row 6							
Clumps	Canes	Total			Affected			Clumps	Canes	Total			Affected			Clumps	Canes	Total			Affected		
		G.L.	D.L.	T.L.	G.L.	D.L.	T.L.			G.L.	D.L.	T.L.	G.L.	D.L.	T.L.			G.L.	D.L.	T.L.	G.L.	D.L.	T.L.
3	13	104	33	137	72	1	73	1	7	84	55	139	68	5	73	2	12	138	63	201	120	30	150
2	6	48	16	64	32	0	32	2	7	75	44	119	52	10	72	2	5	51	27	78	44	12	56
2	7	56	14	70	28	0	28	1	7	69	46	115	49	13	62	1	6	64	88	152	54	17	71
2	4	16	9	25	23	0	23	2	4	41	26	67	28	5	33	1	10	136	55	191	107	15	122
2	5	35	17	52	18	2	20	2	9	106	66	172	62	8	70	2	5	49	25	74	42	5	47
1	4	18	13	31	4	3	7	1	7	77	56	133	26	11	37	2	6	56	38	94	46	1	47
2	12	110	64	174	66	43	109	2	8	84	54	138	39	7	47	2	5	33	34	67	27	7	34
2	10	114	42	156	51	25	76	3	10	100	71	171	36	17	53	3	7	60	45	105	54	9	63
2	7	72	19	91	35	12	47	1	3	32	30	62	16	6	22	3	7	67	49	116	54	5	59
1	7	48	20	68	34	2	36	2	10	110	84	194	37	9	46	3	8	67	44	111	53	6	59
1	6	42	24	66	12	10	22	1	4	46	29	75	20	3	23	3	6	35	28	63	33	2	35
2	6	45	15	60	16	9	25	1	5	50	37	87	21	7	28	3	12	53	45	98	76	16	92
2	8	72	31	103	13	15	28	1	4	39	27	66	8	4	12	2	3	31	16	47	29	4	33
2	8	63	38	101	18	11	29	2	7	52	40	92	12	1	13	3	11	83	53	136	61	7	68
2	8	64	25	89	8	0	8	2	6	53	48	101	13	2	15	3	9	77	34	111	57	6	63
2	6	30	14	44	8	0	8	2	8	74	58	132	14	4	18	1	1	11	6	17	6	0	6
2	8	48	22	70	3	0	3	1	3	19	21	40	7	0	7	1	5	37	24	61	30	2	32
1	5	24	9	33	4	0	4	2	11	88	80	162	22	1	23	2	8	49	89	88	33	3	36
1	4	25	6	31	5	0	5	2	5	44	37	81	14	0	14	2	7	55	39	94	36	0	36
2	5	32	10	42	5	0	5	2	9	87	86	173	22	0	22	2	3	20	18	38	13	0	13
1	5	32	15	47	6	0	6	2	7	80	67	147	13	0	13	1	3	19	6	25	15	2	17
1	4	28	5	33	5	0	5	1	5	45	31	76	8	0	8	2	5	36	14	50	15	1	16
38	148	1126	461	1587	466	133	599	36	146	1455	1093	2548	597	113	710	46	144	1227	790	2017	1005	150	1155

41.39

41.03

81.91

29.86

23.43

49.83

37.74

27.86

57.26



**TABLE**  
*Number of clumps, canes and leaves*

Row 1										Row 2										Row 3									
Units	Clumps	Canes	Total			Affected				Clumps	Canes	Total			Affected				Clumps	Canes	Total			Affected				Clumps	Canes
			G.L.	D.L.	T.L.	G.L.	D.L.	T.L.				G.L.	D.L.	T.L.	G.L.	D.L.	T.L.				G.L.	D.L.	T.L.	G.L.	D.L.	T.L.			
1	1	3	27	8	35	13	4	17	2	6	22	10	32	17	0	17	2	9	40	20	60	13	3	16					
2	1	2	13	3	16	9	0	9	2	6	20	9	29	11	0	11	1	9	11	5	16	7	2	9					
3	2	8	38	15	53	19	7	26	2	8	23	15	38	17	1	18	1	4	14	7	21	7	0	7					
4	2	7	45	15	60	22	6	28	2	11	39	15	54	15	1	16	2	5	32	14	46	16	5	21					
5	1	2	12	4	16	6	1	7	2	8	36	18	54	14	3	17	1	5	34	9	43	23	2	25					
6	2	12	86	30	116	42	1	43	3	8	40	14	54	20	1	21	...	...	...	...	...	...	...	...					
7	1	4	24	10	34	12	2	14	2	7	51	16	67	15	0	15	2	12	101	30	131	35	2	37					
8	1	1	4	3	7	2	3	5	1	3	13	6	19	4	0	4	1	8	87	22	89	14	0	14					
9	2	12	44	29	73	26	1	27	1	11	16	28	88	16	0	16	1	5	53	51	104	17	6	23					
10	2	9	52	52	104	19	0	19	1	3	29	8	37	13	0	13	2	5	83	78	161	16	5	21					
11	2	6	46	38	84	13	1	14	1	5	25	7	32	8	4	12	1	9	87	84	151	26	6	32					
12	2	7	76	57	133	29	2	31	1	3	21	10	31	6	1	7	2	6	64	52	116	20	8	28					
13	2	15	113	98	211	30	7	37	2	12	86	34	120	32	6	38	1	7	42	31	73	18	7	25					
14	1	7	71	58	129	15	5	20	1	2	19	6	25	7	1	8	2	6	61	52	113	18	1	20					
15	1	5	27	22	49	6	0	6	1	4	25	16	41	12	3	15	1	9	75	61	136	29	4	27					
16	3	7	37	38	75	13	0	13	1	4	44	17	61	14	5	19	2	7	73	54	127	20	0	20					
17	2	13	28	72	170	20	15	35	1	7	54	28	82	22	5	27	1	3	38	27	65	9	0	9					
18	2	10	93	68	161	23	4	27	1	3	20	11	31	7	1	8	1	7	82	69	151	20	1	21					
19	1	4	26	23	49	8	4	12	1	6	56	21	77	14	1	15	1	3	30	29	59	8	2	10					
20	1	3	79	64	143	19	9	28	1	9	95	44	139	22	6	28	1	4	47	42	89	15	2	17					
Total	32	136	1011	707	1718	346	72	418	29	126	778	333	1111	286	39	325	26	123	1034	717	1751	326	56	382					

%age affected Green leaves on  
the basis of Green leaves .. 34.22

36.76

31.53

%age affected Green leaves on  
the basis of total leaves .. 20.14

25.74

18.62

%age affected leaves on the  
basis of total leaves .. 24.33

29.25

21.82

## II

(affected and otherwise) that existed in the plot—Plot (2)

Row 4								Row 5								Row 6								
Clumps	Canes	Total			Affected			Canes	Clumps	Total			Affected			Clumps	Canes	Total			Affected			
		G.L.	D.L.	T.L.	G.L.	D.L.	T.L.			G.L.	D.L.	T.L.	G.L.	D.L.	T.L.			G.L.	D.L.	T.L.	G.L.	D.L.	T.L.	
..	..	..	..	..	..	..	..	2	5	61	45	106	12	5	17	1	2	15	9	24	6	0	6	
1	5	47	28	75	14	2	16	1	11	11	90	201	33	15	48	1	7	49	22	71	21	0	21	
2	8	86	39	125	18	19	37	1	3	31	21	52	10	6	16	1	7	41	18	59	21	5	26	
2	7	73	31	104	24	2	26	..	..	..	..	..	..	..	..	1	3	26	10	36	11	3	14	
2	5	61	13	74	20	0	20	1	10	91	81	172	42	7	49	1	6	42	19	61	25	2	27	
4	4	52	22	74	17	0	17	1	4	36	35	71	9	4	13	1	5	20	12	32	18	0	18	
1	3	32	12	44	7	3	10	2	4	45	32	77	21	10	31	2	8	55	22	77	35	1	36	
1	7	96	32	128	18	3	21	1	2	22	19	41	8	1	9	2	5	50	16	66	33	0	33	
1	6	69	36	105	15	3	18	2	6	51	46	97	12	6	18	1	4	40	15	55	14	0	14	
1	3	26	12	38	9	0	9	2	9	81	57	138	20	8	28	1	6	42	11	53	13	0	13	
1	6	48	21	69	11	0	11	1	8	69	66	135	18	7	25	1	4	51	15	66	19	0	19	
2	5	52	27	79	16	1	17	1	4	49	48	87	11	5	16	1	10	57	21	78	18	0	18	
..	..	..	..	..	..	..	..	2	6	62	47	109	20	11	31	1	2	26	7	33	5	0	5	
1	9	57	22	79	18	0	18	1	3	26	23	49	8	4	12	2	6	57	15	72	27	1	28	
1	6	48	24	72	12	0	12	2	3	35	29	64	11	2	13	..	..	..	..	..	..	..	..	
1	7	47	22	69	14	0	14	1	3	32	30	62	9	3	12	1	5	47	15	62	13	3	16	
2	5	40	17	57	10	0	10	1	6	54	48	102	10	6	16	2	8	89	27	116	28	0	28	
1	6	64	25	89	15	0	15	1	4	35	36	71	11	1	12	2	8	77	35	112	33	1	34	
..	..	..	..	..	..	..	..	1	5	46	45	91	12	6	18	..	..	..	..	..	..	..	..	..
1	8	81	31	112	23	1	24	1	5	47	39	86	13	5	18	..	..	..	..	..	..	..	..	..
22	100	979	414	1393	261	34	295	25	101	984	827	1811	290	112	402	22	96	784	289	1073	340	16	356	

26.66

29.47

43.37

18.73

16.01

31.69

21.18

22.20

33.18

Note. G. L. — Green leaves. D. L. — Dry leaves. T. L. — Total leaves.

For the whole plot % age affected  
green leaves = 33.20For the whole plot % age affected  
leaves = 24.59

work. In the first plot, the two border units also have been included—thus making up the total number of units equal to 22 in each row whereas the second plot consists of 20 units only in each of its rows. The number of clumps in each unit, the number of shoots per clump and the number of dry and green leaves (affected and otherwise) of each shoot have been counted and noted in the records. Tables I and II will show the details in these respects. The records of the enumeration of the total puparia on each affected leaf and the measurements of its length and maximum breadth form the actual observations that are subjected to detailed analysis.

The distribution of the puparia in each leaf has also been shown on its sketch drawn roughly on squared paper. The details of all the affected clumps, canes and leaves in the individual units which have been available with the data are entered in Tables III and IV whereas Tables I and II give details of what existed in the plots.

The leaf area has been calculated by multiplying the product of the length and the breadth (at its widest point) of the leaf by 0.7 according to the formula established by Khanna (1935). The population per unit area (square inch) of the affected leaf has been found by dividing the number of puparia on the affected leaf by its area.

### III. SAMPLING PROCEDURE AND THE THEORETICAL BACKGROUND

(a) *Fourfold sampling and the estimation of the required zone-variances.*—The investigation has been divided into two parts, the first part combining the analysis on units, clumps, canes and leaves while the second showing the sufficiency or otherwise of partial examination of a leaf.

The first part is taken to be a case of fourfold Nested (or Hierarchical) sampling with the units, clumps, canes and leaves as the zones of successive orders. The variance of a mean obtained from the nested sampling is a function of the zone variances the estimation of which is, therefore, primarily essential to decide the question of sample size. The theory of Mathematical Expectation affords us the means of finding these effective variances.

We shall, however, require in the present case, results up to the fourfold nested sampling and thus for ready reference the procedure for finding the variances is outlined for fourfold sampling only. Results on the two-fold and three-fold sampling will follow from symmetry:—

A variate in the fourth order zone may be defined as

$$x_{ijkl} = A + b_i + c_{ij} + d_{ijk} + z_{ijkl},$$

TABLE III

*Number of affected clumps, canes and leaves in the Individual Units that are available in the Data Plot (1)*

Row 1			Row 2			Row 3			Row 4			Row 5			Row 6		
U.	Cl.	Leaf	U.	Cl.	Leaf	U.	Cl.	Leaf	U.	Cl.	Leaf	U.	Cl.	Leaf	U.	Cl.	Leaf
1	3	9	74	1	2	11	97	3	1	3	13	70	1	7	72	2	48
2	2	6	67	2	2	6	73	10	2	1	4	15	2	7	71	2	54
3	2	7	87	3	2	5	66	7	3	1	7	28	3	7	62	1	70
4	1	6	43	4	2	7	64	12	4	2	4	23	4	7	33	2	115
5	2	1	77	5	2	11	82	6	4	2	3	19	5	9	70	1	47
6	1	10	95	6	1	3	33	9	6	1	4	7	7	7	37	2	46
7	2	8	94	7	1	3	41	5	7	2	12	88	8	8	46	5	32
8	1	5	59	8	1	4	47	2	8	2	10	68	7	9	51	3	61
9	3	10	107	9	2	9	76	2	9	1	4	31	9	3	22	3	56
10	1	9	57	10	2	7	63	8	10	1	6	28	10	3	9	3	58
11	2	8	58	11	1	6	60	12	11	1	6	20	11	..	..	2	35
12	1	6	40	12	2	9	106	5	12	2	6	25	12	5	27	3	89
13	3	7	49	13	2	7	76	3	13	2	8	21	13	1	4	1	32
14	3	11	96	14	2	13	139	4	14	2	8	26	14	4	13	3	11
15	2	7	51	15	1	9	69	15	15	1	2	2	15	1	5	2	8
16	2	6	52	16	..	..	..	2	16	2	4	2	16	2	7	1	6
17	1	5	41	17	2	8	88	1	17	1	3	3	17	..	..	1	32
18	1	3	14	18	2	7	46	1	18	1	3	4	18	8	22	2	36
19	2	9	57	19	2	7	82	1	19	1	3	5	19	2	14	2	7
20	2	8	51	20	1	3	38	2	20	2	3	5	20	8	22	2	13
21	2	9	51	21	2	9	88	5	21	1	4	6	21	6	12	1	17
22	2	5	33	22	1	6	75	6	22	..	..	..	22	3	8	2	16
Total	42	161	1353	21	35	150	1529	39	21	32	117	502	20	124	672	43	1030

Average per unit: 1.91 7.32 61.50 1.67 7.14 72.81 1.77 6.75 53.59 1.52 5.57 23.90 1.60 6.20 33.60 1.95 6.27 46.81

TABLE IV

*Number of the affected clumps, canes and leaves in the Individual Units that are available in the Data Plot (2)*

Row 1				Row 2				Row 3				Row 4				Row 5				Row 6			
U.	Cl.	Cane	Leaf	U.	Cl.	Cane	Leaf	U.	Cl.	Cane	Leaf	U.	Cl.	Cane	Leaf	U.	Cl.	Cane	Leaf	U.	Cl.	Cane	Leaf
1	1	3	17	1	1	2	17	1	2	7	15	..	1	1	4	17	1	2	6	1	1	2	6
2	1	2	8	2	2	5	11	2	3	2	9	..	2	1	11	48	2	7	16	2	1	7	16
3	2	7	26	3	1	5	16	3	4	8	7	5	3	1	3	16	3	1	25	3	1	7	25
4	2	7	27	4	2	7	16	4	2	7	18	2	2	..	..	..	4	1	13	4	1	3	13
5	5	2	7	5	2	6	16	5	5	5	25	5	2	1	10	48	5	1	19	5	1	5	19
6	2	12	43	6	4	14	14	6	6	4	17	3	2	4	4	13	6	1	17	6	1	5	17
7	1	1	14	7	2	6	15	7	1	3	..	1	1	1	31	31	7	1	24	7	1	5	24
8	1	1	5	8	..	..	..	8	1	7	14	7	1	2	9	9	8	2	33	8	2	5	33
9	2	10	26	9	1	5	11	9	1	5	23	1	1	2	5	18	9	1	13	9	1	4	13
10	2	7	19	10	1	3	12	10	2	5	21	3	1	8	27	10	1	5	13	10	1	5	13
11	2	5	14	11	1	3	11	11	1	9	31	5	1	8	23	11	1	4	19	11	1	4	19
12	2	7	31	12	1	3	7	12	1	6	28	5	2	4	16	12	1	6	18	12	1	2	18
13	2	12	36	13	2	10	38	13	1	7	22	..	13	2	6	28	13	1	5	13	1	2	5
14	1	6	20	14	1	2	8	14	1	6	20	6	1	3	12	14	2	6	28	14	2	6	28
15	1	3	6	15	1	4	15	15	1	9	27	..	14	1	3	12	15	..	..	15	..	..	..
16	3	6	13	16	1	4	18	16	2	7	20	3	1	3	12	16	1	5	16	1	5	5	16
17	2	10	32	17	1	6	27	17	1	3	9	4	2	1	5	12	17	2	28	17	2	8	28
18	2	9	27	18	1	3	8	18	1	7	20	6	1	4	12	18	2	8	34	18	2	8	34
19	1	3	12	19	1	6	10	19	1	8	10	..	19	1	5	16	19	..	..	19	..	..	..
20	1	3	13	20	1	9	28	20	1	4	17	8	1	5	18	20	1	..	..	20	..	..	..
Total	2032	119	396	19	27	96	298	19	23	111	373	94	22	23	395	17	21	87	327	17	21	87	327

Average per unit : 1.60 5.95 19.80 1.42 5.05 15.68 1.21 5.84 19.63 1.29 5.53 16.53 1.21 5.05 20.79 1.24 5.12 19.24

Note.— U = Units.

Cl = Clumps.

where satisfying the conditions of homoscedasticity we have

$$E(b_i) = 0; E(b_i)^2 = \sigma_1^2; E(c_{ij}) = 0; E(c_{ij})^2 = \sigma_2^2;$$

$$E(d_{ijk}) = 0; E(d_{ijk})^2 = \sigma_3^2; E(z_{ijkl}) = 0; E(z_{ijkl})^2 = \sigma_4^2.$$

From these relations can be found the expectation of the mean and its variance which are respectively:—

$$(1) E(X \dots) = A$$

$$\text{and } (2) V(X \dots) = \frac{\sum p_i^2}{p^2} \sigma_1^2 + \frac{\sum_i \sum_j p_{ij}^2}{p^2} \sigma_2^2 + \frac{\sum_i \sum_j \sum_k p_{ijk}^2}{p^2} \sigma_3^2 + \frac{1}{p} \sigma_4^2.$$

where  $p$  is the total number of variates and  $p_i$ ,  $p_{ij}$  and  $p_{ijk}$  are the number of the fourth order variates in the selected units of the first, second and third order zones respectively. In equal case (that is where the number of variates is equal from unit to unit in a zone), the formula (2) reduces to

$$V(X \dots) = \frac{\sigma_1^2}{t} + \frac{\sigma_2^2}{n} + \frac{\sigma_3^2}{m} + \frac{\sigma_4^2}{p}$$

where  $t$ ,  $n$  and  $m$  are the total number of variates in the successive zones.

Let  $v_1$ ,  $v_2$ ,  $v_3$  and  $v_4$  represent the successive variances of the zones in the structure of the analysis of variance.

Then  $v_4$  will be the unbiased estimate of  $\sigma_4^2$ .

$$(m - n) v_3 \quad \text{do} \quad \text{do} \quad \text{of} \left\{ p - \sum_i \sum_j \frac{\sum_k p_{ijk}^2}{p_{ij}} \right\} \sigma_3^2 + (m - n) \sigma_4^2.$$

$$(n - t) v_2 \quad \text{do} \quad \text{do} \quad \text{of} \left\{ p - \sum_i \frac{\sum_j p_{ij}^2}{p_i} \right\} \sigma_2^2 \\ + \left\{ \sum_i \sum_j \frac{\sum_k p_{ijk}^2}{p_{ij}} - \sum_i \frac{\sum_j \sum_k p_{ijk}^2}{p_i} \right\} \sigma_3^2 + (n - t) \sigma_4^2.$$

$$(t - 1) v_1 \quad \text{do} \quad \text{do} \quad \text{of} \left\{ p - \frac{\sum_i p_i^2}{p} \right\} \sigma_1^2 \\ + \left\{ \sum_i \frac{\sum_j p_{ij}^2}{p_i} - \frac{\sum_i \sum_j p_{ij}^2}{p} \right\} \sigma_2^2 + \left\{ \sum_i \frac{\sum_j \sum_k p_{ijk}^2}{p_i} - \frac{\sum_i \sum_j \sum_k p_{ijk}^2}{p} \right\} \sigma_3^2 + (t - 1) \sigma_4^2.$$

It should, however, be remembered here that the question of estimating the  $\sigma$ 's arises only when the null-hypothesis is found to be not true.

(b) *Calculation of the sampling efficiency percentages so far as sampling in a unit is concerned.*—The random selection in the number of units having been made, the next question that arises is with regard to further sampling in the unit itself. The desirability of further sampling in clumps, canes and leaves from out of the selected units has to be tested on the merit of its efficiency. Lesser number of clumps in a unit, fewer canes in a clump and fewer

leaves in a cane will be preferred to complete enumeration only when the efficiency of the proposed sampling or the *information* afforded by it is high. So far as sampling in a unit is concerned, efficiency percentage may be defined as

$$\frac{100 \left( \sigma_1^2 + \frac{\sum_i \sum_j p_{ij}^2}{\sum_i p_i^2} \sigma_2^2 + \frac{\sum_i \sum_j \sum_k p_{ijk}^2}{\sum_i p_i^2} \sigma_3^2 + \frac{p}{\sum_i p_i^2} \sigma_4^2 \right)}{\sigma_1^2 + \frac{t}{n} \sigma_2^2 + \frac{t}{m} \sigma_3^2 + \frac{t}{p} \sigma_4^2}$$

The formula explains for itself what has been sought to be conveyed by sampling efficiency. The numerator in the formula is the mean variance in the unit where the  $\sigma$ 's are substituted for by the respective estimated zone variances and the coefficients are based on the total number of variates that existed in the unit. The denominator is the mean variance in the unit based on the adopted sampling. This formula therefore takes account simultaneously of the number of variates and the respective variances and shows how near has been the size of the adopted sampling to the subpopulation existing in the unit.

#### IV. FORMATION OF THE PATCHES AND SAMPLING IN THE PLOTS

Charts (1) and (2) show the distribution of the incidence over the 2 plots. It is quite interesting to see the way in which whitefly has distributed itself in different patches of intensity. Considering the following classifications for low, medium and severe incidence, the three grades of intensity are fairly distinct from one another except the formation of low patch in plot 2.

Grade				Number of puparia per square inch of affected leaves	
Low	..	..	..	..	Less than 2.00
Medium	..	..	..	..	Between 2.00 and 5.51
Severe	..	..	..	..	Above 5.51

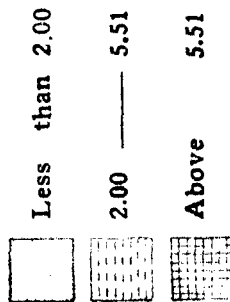
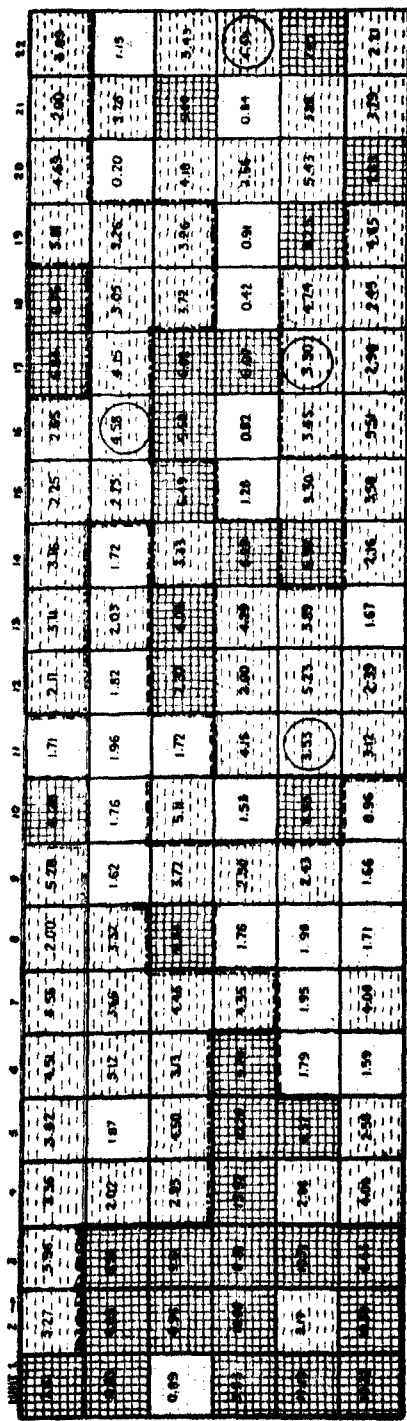
No puparia counts were available for 4 units in plot (1) and 9 units in plot (2) the incidence figures for which have been interpolated. The occurrence of two interpolated values in the low patch of plot (2) has partly impaired its well marked formation.

*Sampling in plot (1).*—Each of the patches so formed has been subjected to nested sampling and in each case 10 units have been chosen at random as the first order zones. Besides these, one more analysis in each plot has been done where 10 units have been selected at random out of the entire plot irrespective of the patches. This analysis will be referred to as the pooled analysis. In case of the low-patch in plot (1), the three units serving as the connecting links have not been considered for random selection.

# PLOT No. 1

## UNITS SHOWN ACCORDING TO THE INTENSITY OF INCIDENCE

Puparia per Sqr. Inch of Leaf Area



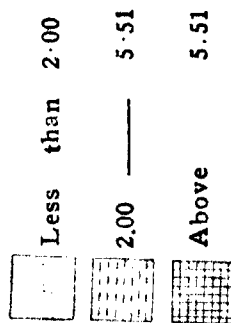
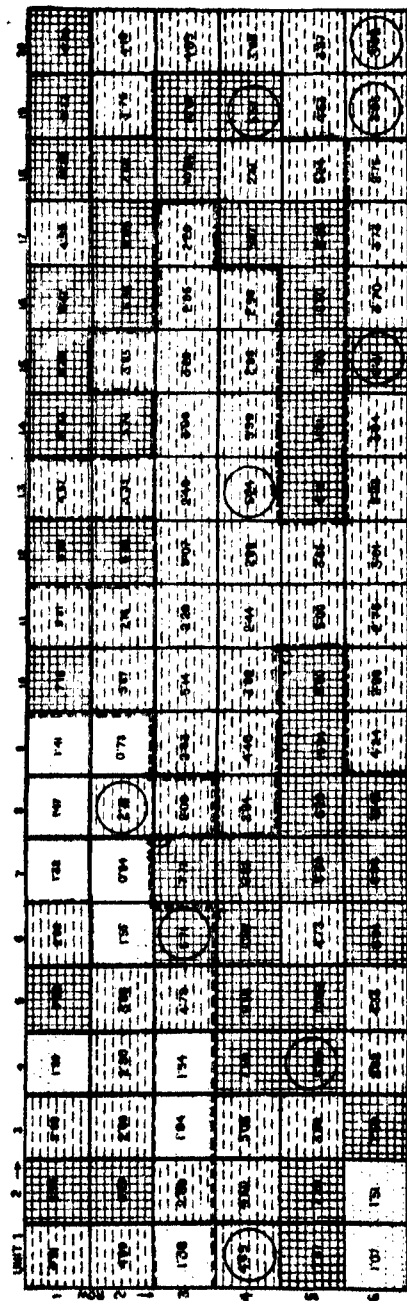




# PLOT No. 2

## UNITS SHOWN ACCORDING TO THE INTENSITY OF INCIDENCE

Puparia per Sqr. Inch of Leaf Area





For the estimation of the zone variances and consequent analysis, two clumps per unit, two canes per clump and five affected leaves per cane have been successively selected at random in plot (1) in the 'low' and 'severe' patches. Owing to insufficient number of clumps per unit in the medium II-patch, only one clump at random from each unit has been included in the analysis. Again since the affected leaves per cane in the medium I-patch have not been sufficiently more than five in general, only 3 leaves per cane instead of five have been taken in this case to make allowance for sufficient choice in randomisation. In the pooled analysis, however, all clumps, all canes and all leaves have been included in the analysis for the estimation of the zone-variances.

*Sampling in plot (2).*—It will be apparent from Tables I and II that the crop growth in plot (2) is much poorer than that of plot (1) in respect of clumps per unit, canes per clump, leaves per cane and even in respect of the magnitude of leaf area. On account of this, sufficient choice for random selection of clumps, canes and leaves in the abovementioned proportions is not possible in plot (2). So in plot (2), all clumps, canes and leaves have been included in the analysis of variance to ensure sufficient degrees of freedom.

*The question of transformation.*—The ultimate variate used here is the mean number of puparia per square inch of the leaf surface, calculated on the basis of affected leaves. The question of some sort of transformation

### Variances due to different zones

(With and without transformation)

#### Plot 1

	Mean with S.E.	$\sigma_m$ exp. as % of mean	$\sigma_1^2$	$\sigma_1^2$ as % of total	$\sigma_2^2$	$\sigma_2^2$ as % of total	$\sigma_3^2$	$\sigma_3^2$ as % of total	$\sigma_4^2$	$\sigma_4^2$ as % of total
Severe	7.9131	8.1258	12.7883	9.8168	6.6294	5.0890	11.8286	9.0800	99.0236	76.0142
	$\pm 0.643$	2.3462	0.0150	4.9293	0.0049	1.6103	0.0282	0.2672	0.2562	84.1932
Medium	$\pm 0.061$	3.58	0.0438	0.4164	0.00	0.00	0.00	0.00	10.4747	99.5836
	$\pm 0.295$	8.2402	0.0085	4.6858	0.0053	2.9217	0.00	0.00	0.1676	92.3925
Low	$\pm 0.0026$	0.1005	0.00	0.00	0.00	0.00	0.3414	11.2988	2.6807	88.7032
	1.6823	6.8953	0.00	0.00	0.00	0.00	0.0106	6.81	0.1430	91.84
	$\pm 0.116$	0.0923	0.00	0.00	0.0021	1.35	0.0106	6.81	0.1430	91.84
	2.058	0.0923	0.00	0.00	0.0021	1.35	0.0106	6.81	0.1430	91.84
	$\pm 0.0019$									

*N.B.*—The upper figures are the results of the analysis of the actual incidence.

The lower figures are the results of the analysis of logarithm of (incidence  $\times$  100).

being needed for this variate has been examined and it is found that transformation is not essential in this case. Analysis of variance has been actually done in plot (1) with logarithmic transformation after multiplying each incidence figure by 100. The results are reproduced above.

It will be noticed in the above statement that leaves take away the maximum variation in both the cases and that there is also a distinct similarity in the distribution of the percentage variations in the other zones. There is therefore no point in making use of the transformation as the accruing advantage will not be of a considerably high order offsetting the extra inconvenience to be encountered in handling the transformed material and in interpreting the results in terms of the original units. Though not actual percentages the mean puparia per square inch very much resemble percentage figures where the necessity of a transformation is universally recognized. Even in such a domain transformation is not always needed. It is well known that no transformation would need to be made if the range in percentages is between about 25 and 75.

#### V. DISCUSSION OF THE RESULTS

(a) *High variability of the variate; largest variation due to leaves.*—Tables V and VI give the results of the analysis of variance in the different patches as well as in the pooled one. The estimates of the zone-variances in original and as percentages of the total variation are given at the bottom of Tables VII and VIII where  $E(\sigma_1^2)$ ,  $E(\sigma_2^2)$ ,  $E(\sigma_3^2)$  and  $E(\sigma_4^2)$  represent respectively the estimates of variances in units, clumps, canes and leaves.

A high degree of symmetry, however, exists in the two plots as will be noticed in the largest percentage variations due to leaves as also in the location of zero variances in some patches.  $\sigma_2^2$  and  $\sigma_3^2$  are zero respectively in the low and medium patches of both the plots.  $\sigma_3^2$  in the medium II—patch of plot (1) expressed as percentage of the total variation comes out to be 0.28 which also is negligibly small and may be taken to be of the zero order for all practical purposes. The percentage variation of  $\sigma_4^2$  in the pooled analysis of plot (2) is 0 whereas the corresponding figure in plot (1) is 1.69 which also is obviously a small percentage.

In Tables VII and VIII will be found in respect of each type of patch, the errors expressed as percentages of the means corresponding to the different combinations of clumps, canes and leaves to be selected in a unit. The first three columns will show the selected combination, the next column gives the number of total leaves arising out of the combination in a unit and this expressed as percentage of the total affected leaves of the unit is entered in the adjacent column. The last column under each category will show the

TABLE V. Results of the analysis of variance in the different patches—Plot (1)

Due to	Low infested zone					Medium I					Medium II				
	D.F.	S.S.	M.S.	Ratio	D.F.	S.S.	M.S.	Ratio	D.F.	S.S.	M.S.	Ratio	D.F.	S.S.	Ratio
Between Units	9	17.8342	(V <sub>1</sub> ) 1.9594	0.73	9	99.0047	11.0050	1.05	..	..	..	..	..	..	..
Between clumps within a unit	10	40.7279	(V <sub>2</sub> ) 4.0738	1.52	10	79.0301	7.9031	0.76	..	..	..	..	..	..	..
Between clumps	19	58.5721	3.0827	1.15	19	178.0354	9.3703	0.90	9	74.9093	8.3233	1.637	9	74.9093	1.637
Between canes within a clump	20	87.7590	(V <sub>2</sub> ) 4.3830	1.64	20	184.9874	9.2494	0.88	10	51.5952	5.1595	1.016	10	51.5952	1.016
Between canes	39	146.3311	3.7521	1.40	39	363.0228	9.3082	0.89	19	126.5045	6.6581	1.390	19	126.5045	1.390
Between leaves within a cane	160	428.9148	(V <sub>4</sub> ) 2.6807	..	80	837.9678	10.4746	..	80	406.7572	5.0845	..	80	406.7572	..
Total	199	575.2459	..	..	119	1200.9906	..	..	99	533.2617	..	..	99	533.2617	..

	Severe					Pooled				
	D.F.	S.S.	M.S.	Ratio	D.F.	S.S.	M.S.	Ratio	D.F.	Ratio
Between units	9	4322.0375	(V <sub>1</sub> ) 480.2464	4.85	9	3020.8229	335.6476	9.054	9	9.054
Between clumps within a unit	10	2244.6077	(V <sub>2</sub> ) 224.4608	2.27	10	762.1847	76.2185	2.056	10	2.056
Between clumps	19	6566.6452	345.6129	3.49	19	3782.0076	199.1057	5.371	19	5.371
Between canes within a clump	20	3163.3303	(V <sub>3</sub> ) 158.1665	1.66	44	2499.6997	56.8114	1.532	44	1.532
Between canes	39	9729.9755	249.4865	2.52	63	6282.7073	99.7255	2.690	63	2.690
Between leaves within a cane	160	15843.7690	(V <sub>4</sub> ) 99.0236	..	488	18090.9912	37.0717	..	488	..
Total	199	25573.7445	..	..	551	24373.6985	..	..	551	..

TABLE VI. Results of the Analysis of variance in the different patches—Plot (2)

Due to	Low				Medium				Severe I			
	D.F.	S.S.	M.S.	Ratio	D.F.	S.S.	M.S.	Ratio	D.F.	S.S.	M.S.	Ratio
Between units	9	300.5585	33.3933 (V <sub>1</sub> )	12.146	9	111.5204	12.3911	1.426	9	720.1908	80.0213	3.152
Between clumps within a unit	5	7.8973	1.5795 (V <sub>2</sub> )	0.574	2	38.9395	19.4697	2.242	3	232.0041	77.3347	3.047
Between clumps	14	30844558	22.0326	8.013	11	150.4599	13.6782	1.574	12	952.1949	79.3496	3.126
Between canes within a clump	34	129.6351	3.8128 (V <sub>3</sub> )	1.367	40	301.5856	7.5397	0.863	29	424.1010	14.6242	0.576
Between canes	48	438.0909	9.1268	3.319	51	452.0485	8.8637	1.020	41	1376.2959	33.5682	1.322
Between leaves within a cane	100	274.9634	2.7496 (V <sub>4</sub> )	..	120	102.2929	8.6857	..	108	2741.2032	25.3815	..
Total	148	713.0543	..	..	171	1494.3314	..	..	149	4117.4991	..	..

Due to	Severe II				Pooled			
	D.F.	S.S.	M.S.	Ratio	D.F.	S.S.	M.S.	Ratio
Between units	9	1363.6460	151.5162 (V <sub>1</sub> )	4.988	9	887.9436	98.6604	6.115
Between clumps within unit	2	7.4006	3.7003 (V <sub>2</sub> )	0.122	3	66.2994	22.0998	1.369
Between clumps	11	1371.0466	124.6406 (V <sub>3</sub> )	4.102	12	954.2430	79.5202	4.928
Between canes within a clump	38	1739.8522	45.7855 (V <sub>4</sub> )	1.507	25	635.6103	25.4244	1.575
Between canes	49	3110.8988	63.4877 (V <sub>5</sub> )	2.090	37	1589.8533	42.9690	2.663
Between leaves within a cane	154	4678.2193	30.3780 (V <sub>6</sub> )	..	91	1468.2398	76.1345	..
Total	203	7789.1181	..	..	128	3058.0921	..	..

TABLE VII. Error as percentage of the Mean in the different types of sampling with the corresponding sampling efficiencies (in percentage) so far as sampling in a unit is concerned and the estimates of the zone-variances Plot (1)

Low				Medium I				Medium II				Severe				Pooled							
Clump	Cane	Sampling Combination	No. of leaves	%age leaves sampled	Error as %age of the mean	Sampling efficiency as %age	Error as %age of the mean	%age leaves sampled	Sampling efficiency as %age	No. of leaves	%age leaves sampled	Error as %age of the mean	Sampling efficiency as %age	Error as %age of the mean	%age leaves sampled	Sampling efficiency as %age	Error as %age of the mean	Sampling efficiency as %age	Error as %age of the mean	%age leaves sampled	Sampling efficiency as %age		
1	1	1	1	1.28	32.69	2.29	28.77	2.04	2.08	1	1	1.49	30.38	7.69	1	1.24	45.62	14.27	1.81	41.49	13.50		
1	1	1	3	3.85	20.21	5.60	16.48	6.11	6.26	1	3	4.48	18.47	20.55	3	3.71	31.85	28.92	5.43	27.79	30.04		
1	1	1	5	8.42	17.83	7.89	..	..	..	1	5	7.46	15.19	30.88	5	6.18	28.43	36.41	9.06	24.07	39.78		
1	1	2	1	2.57	23.18	4.58	20.39	4.07	4.15	2	1	2.99	22.17	14.53	2	2.47	34.50	24.83	3.62	31.12	24.02		
1	1	2	3	7.70	14.86	11.21	11.73	12.22	12.24	2	3	8.96	13.96	35.55	6	7.42	25.91	44.43	10.87	22.11	47.07		
1	1	2	5	12.84	12.48	15.77	..	..	..	2	5	14.92	11.90	50.02	10	12.36	23.76	52.75	18.12	19.96	58.25		
2	1	1	1	2.57	23.18	4.58	20.39	4.07	4.15	..	..	..	..	..	..	..	..	..	..	..	..		
2	1	1	3	7.70	14.86	11.21	11.73	12.22	12.24	..	..	..	..	..	..	..	..	..	..	..	..		
2	1	1	5	12.84	12.48	15.77	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
2	2	1	1	5.13	16.05	9.16	14.53	8.15	8.23	..	..	..	..	..	..	..	..	..	..	..	..		
2	2	1	3	15.40	10.70	22.42	8.38	24.44	23.90	..	..	..	..	..	..	..	..	..	..	..	..		
2	2	1	5	25.67	8.92	31.54	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
				5.35			4.47						7.39				19.08			16.83			
Low					..	0	0	0	0	0	0	0	2.6807	0	0	0	11.30			88.70			
Medium I					..	0.0438	0	0	0	0	0	10.4747	4.16	4.16	0	0	0	0			95.84		
Medium II					..	8.3164	8.3164	0.0180	0.0180	5.0845	5.0845	5.84	5.84	0.28	0.28	5.84	5.84	0.28	0.28			93.88	
Severe					..	12.7883	6.6294	11.8286	11.8286	99.0236	9.82	9.82	5.09	5.09	9.08	9.08	9.08	9.08			76.01		
Pooled					..	4.8159	0.7584	2.2551	2.2551	37.0717	10.73	10.73	1.69	1.69	5.02	5.02	5.02	5.02			92.56		



TABLE VIII. Error as percentage of the mean in the different types of sampling with the corresponding sampling efficiencies (in percentage) so far as sampling in a unit is concerned and the estimates of the zone-variances—Plot (2)

Low			Medium I			Severe I			Severe II			Pooled		
Sampling combinations	No. of leaves	%age leaves sampled	Error as %age of the mean	Sampling efficiency as %age	%age leaves sampled	Error as %age of the mean	Sampling efficiency as %age	%age leaves sampled	Error as %age of the mean	Sampling efficiency as %age	%age leaves sampled	Error as %age of the mean	Sampling efficiency as %age	
														Clamp
1	1	6.71	36.86	44.01	5.81	27.22	13.28	6.67	27.95	24.47	11.90	26.31	18.07	
1	1	20.13	29.48	68.76	17.44	17.42	32.50	20.00	19.69	49.23	14.71	18.36	37.08	
1	1	33.56	27.77	77.48	29.07	14.67	45.74	35.33	17.57	61.77	24.51	16.37	46.96	
1	1	13.42	30.66	63.57	11.63	20.31	23.83	13.33	22.03	39.25	9.80	19.86	31.90	
1	1	40.27	26.38	85.91	34.88	13.90	50.93	40.00	17.00	65.98	29.41	14.66	58.28	
1	1	67.11	25.45	92.40	58.14	12.22	65.88	66.67	15.80	76.37	49.02	13.41	69.58	
2	1	13.42	30.66	63.58	11.63	19.24	26.55	13.33	19.76	48.84	9.80	19.86	31.90	
2	1	40.27	26.38	85.91	34.88	12.33	64.99	40.03	13.92	98.46	29.41	14.66	58.28	
2	1	67.11	25.45	92.40	58.14	10.38	91.48	66.67	12.43	78.42	49.02	13.41	69.58	
2	1	26.85	27.05	81.75	23.26	14.37	47.72	26.67	15.58	78.42	19.61	15.56	51.68	
2	2	80.54	24.68	98.15	69.77	9.83	72.02	80.00	12.02	..	58.82	12.41	81.60	
2	2	..	..	..	..	..	..	..	..	..	98.04	11.67	92.28	
			25.29	11.07			12.25			12.54			20.21	

Low Medium Severe I Severe II Pooled	E (61 <sup>2</sup> )		E (62 <sup>2</sup> )		E (63 <sup>2</sup> )		E (64 <sup>2</sup> )		Variances		Expressed		As %age Variations	
	..		0		0		2.7496		38.45		0		7.56	
Low	1.0584		1.1038		0.3848		8.6857		0		11.28		0	
Medium	0		8.2031		0		25.3815		0		24.43		0	
Severe I	0		0		0		3.8748		0		0		9.81	
Severe II	5.2465		0		3.8748		30.3780		13.28		0		78.91	
Pooled	5.6889		0		2.8302		16.1345		23.07		0		11.48	

percentages of sampling efficiency corresponding to the individual types of combination, efficiency being calculated on the same procedure as outlined in Section 3 (b). While considering the results in plot (2), it should be remembered that though the average number of canes per unit is mostly greater than 4, the average number of clumps per unit is hardly equal to 1.5. Combination with two clumps per unit, therefore, will necessarily have its limitation. The size of the adopted sampling in many of the combinations will cover nearly all the available clumps and as such will be nearer the total elements available in the units from which samples were drawn. This is a reason why efficiency percentage in plot (2) has sometimes been very high. The line corresponding to the combination of 2 clumps  $\times$  2 canes  $\times$  5 leaves has been kept blank since the average number of leaves per unit is in no case equal to 20.

It will be noticed in Tables VII and VIII that the largest variation is due to leaves. In plot (1) the variation ranges from 76.01 per cent. to 95.84 per cent. while in plot (2) it ranges from 53.99 per cent. to 88.72 per cent. This obviously indicates that the totality of leaves is the most predominant factor in the effective reduction of the error of the estimate. The error percentage column will give an idea as to how the error falls off with the increase in the number of leaves. It demonstrates, therefore, the relative merit of the different types of sampling combination so far as reducing the error is concerned.

The contributions that can be made by zones other than leaves have fully reflected themselves in the errors of the different sampling combinations. The trend of errors under these combinations which have been sufficient in number would throw light on the potentialities of the different zones in the error reduction.

If the estimated variances are taken as truly representative of the zone variances, reduction of the error may be carried beyond the size of the sample and extended up to the total number of leaves, canes and clumps that were available in the 10 units. The last (isolated) figure shown below the error percentage column represents this minimum reducible error in the different patches. The lowest of these figures in plot (1) is 4.47% and occurs in the medium 1-patch whereas the highest value 19.08% occurs in the severe patch. In the pooled analysis, the minimum error has come out to be 16.83% of the mean. These figures amply demonstrate the highly variable nature of whitefly incidence from leaf to leaf. That the variation is really very wide will be clear from the fact that in case of 19.08% and

16.83% that is in the severe and the pooled patches the number of leaves available in the units are respectively 809 and 552.

Owing to less number of leaves occurring in plot (2), the minimum reducible error in this plot has been of still higher order. The sample size in leaves varies from 129 in the pooled analysis to 204 in the severe II-patch, the corresponding minimum errors being 20.21% and 12.54%, the lowest error being 11.07% in the medium patch for a sample size of 172 leaves. These minimum errors are more important in plot (2) in so far as they will fix up the upper limits of the sampling combinations, to be adopted. In the error column no figure should be less than this minimum error. In case where the minimum error is greater than any figure in the error column, the corresponding sampling combinations is not valid in the particular instance but it has none the less been entered to show the extent and possibility of error reduction.

(b) *Varied sampling requirements under different grades of intensity.*—Examination of Table VII—particularly the percentage variations of the zones will reveal that when sampling has to be done in a region of low or medium infestation, the sampling selection may be confined to leaves alone. Sampling need not be of the nested type. That is to say, the affected leaves of all the clumps (or canes) in a unit may alone be subjected to random selection without any consideration to see whether or not the leaves drawn represent all the clumps in a unit or all the canes in a clump. In such a case, the variation due to leaves is the only variation that matters. But in case of severe infestation or in cases where it is fairly high (represented by the type of pooled analysis), sampling procedure should necessarily be of the Nested-type so that the clumps and canes in a unit may be equitably represented. Percentage variations of the different zones as given in Table VIII also exhibit the same results in respect of plot (2) except in the low-patch where the results are in favour of Nested sampling. It has been already taken into notice that the formation of the low patch in plot (2) has not been well enough nor has it been sufficiently large.

Another important feature that has come out prominently is the occurrence of the lowest error under the medium patch in both the plots. Therefore, for a required error percentage, the sample size to be needed in such a region will be comparatively small. Fixing the limit of the error to be near about 15% of the mean, the following table is prepared to furnish the information on the relative requirements of the sample size under different grades of infestation. Percentages of leaves required in the medium patch to those required on the average in the severe and pooled patches form roughly about 23 per cent. in plot (2) and 11 per cent. in plot (1).

Classification	Plot 1					Plot 2				
	Unit	Clump	Canes	Leaf	Error %	Unit	Clump	Canes	Leaf	Error %
Low	10	2	1	3	14.86	15	2	2	5	14.98
Medium (I)	10	2	2	1	14.53	10	1	1	5	14.67
Medium (II)	10	1	1	5	15.19	..	..	..	..	..
Severe (I)	15	2	3	6	14.89	10	2	2	1	15.58
Severe (II)	..	..	..	..	..	10	2	1	3	14.66
Pooled	13	2	2	5	14.98	15	2	2	5	14.98

(c) *Percentage area to be sampled.*—The 3 ft. units of the plots served as the first order zones. Random selection in the units gives representation to the spread of the plot area. For the sake of uniformity, 10 units have been selected from each patch as well as in the pooled analysis which covering as it does the whole plot, will be of practical interest. Table IX will show that except for Medium II-patch in plot (1) the difference between the whole-patch mean and the one obtained from the sample is fairly within the limits of the standard error. In the pooled analysis ten 3 ft. units form respectively 7.81% and 9.01% of the total number of units available in the plots. The corresponding error percentages are, however, 16.83 and 20.21 in the two cases. If 15 units (about 13.51% of the total units) be selected in plot (2) with the same combination, (2 clumps  $\times$  2 canes  $\times$  5 leaves per unit), the error will reduce from 20.21 per cent. to about 15 per cent. whereas in plot (1) 13 units (forming about 10.16%) will bring down the error from 16.83 per cent. to roughly about 15%. Further reduction in the error per cent. will necessitate more units to be taken in the sample. Variability of incidence of white-fly is of such a magnitude that if the estimate is required within an error of 5 per cent., almost all the units of the plot (1/40th acre) will have to be sampled. 5 per cent. in error therefore will appear to be too much to expect in practice in a plot of 1/40th acre. 10 per cent. of units from an  $\frac{1}{4}$ th acre plot is likely to reduce the error in estimation to about 5 per cent. provided, however, the variation in incidence in the larger plot (such as  $\frac{1}{4}$ th acre) does not correspondingly increase in dimension.

In plot (1), 20 leaves (2 clumps  $\times$  2 canes  $\times$  5 leaves) in the pooled analysis have formed about 17 per cent. of the affected leaves. In case where the combination, 2 clumps  $\times$  2 canes  $\times$  5 leaves is not available in a unit in the plot, about 20 leaves equitably representing the clumps and canes should be taken. Alternatively, about 17–20 per cent. of the affected leaves (*i.e.*, one in every five) per unit should be taken if this gives larger number of leaves in the sample.

TABLE IX. *Sample-mean and the mean from the whole patch—Plot (1)*

Plot I							Plot II				
Grades	Mean for the whole patch	Sample mean	Diff. as %age of the mean	S. E. as %age of the mean	%age units sampled	Grades	Mean for the whole patch	Sample mean	Diff. as %age of the mean	S. E. as %age of the mean	%age units sampled
Low	..	1.68	11.31	8.92	58.82	Low	..	1.94	6.19	23.39	83.33
Medium I	..	3.53	1.40	8.38	29.41	Medium	..	3.63	3.58	11.67	29.41
Medium II	..	2.44	50.83	11.90	55.56	Severe I	..	6.56	14.33	15.25	47.61
Severe	..	7.91	4.55	15.69	47.62	Severe II	..	7.55	1.85	12.54	45.45
Pooled	..	5.11	14.29	16.83	7.81	Pooled	..	4.63	9.72	20.21	9.01

It may be pointed out that the sampling procedure evolved in this note would strictly apply to a plot of 1/40th acre in size, but the contribution to error made by plots or by any other upper hierarchies will perhaps be small for practical purposes as the maximum of the total variation has been consumed by leaves in all the patches except in the low patch of plot (I). Study of the material divided into patches of low, medium and severe infestation has thrown sufficient light on the extent of variations that might possibly exist in a bigger plot or field where the infestation has to be either low or medium or severe. Results of investigations made in this direction will be furnished in a subsequent communication.

#### VI. RESULTS ON THE NUMBER OF INCH-UNITS TO BE SELECTED IN A LEAF

It now remains to see whether partial enumeration of a leaf will serve the purpose of estimation with fair accuracy. It is difficult indeed to select random square inch units from the area of the leaf since the breadth of the leaf is not uniform throughout. If a leaf is divided into inch units along its length each unit covering the entire breadth of that portion of the leaf, the units so formed will more easily lend themselves to random selection. If these units be chosen at random in sufficient number, the total area of these units may be calculated for practical purposes exactly in the same way as the net area of the leaf itself was found for working out the puparia per square inch.

For the purpose of estimating the variances in the present analysis, two leaves per unit and 10 random inch-units per leaf have been selected at random from each of the regions of low, medium and severe infestation.

Table X will show the estimates of the variances between leaves as well as within leaves. The last line in the table gives the standard error expressed as percentage of the mean; the sample in each case consists of 20 leaves and 200 inch units in all.

The calculation of the puparia per square inch of the leaf-area has already been found out. Now, the puparia per square inch of the leaf has been found from consideration of the area of these random inch-units, which are 10 in number in each case. The net area of these 10 units has, however, been found by the same process as was used in finding out the area of the leaf itself. The puparia per square inch of the leaf worked as such as well as the puparia found from consideration of the entire leaf area, have been shown side by side in Table X. The difference in between these two estimates has been nowhere significant as revealed by the 't' tests. It, therefore, justifies the method used in finding the net area of these random units besides showing that 10 random units are sufficient in giving an estimate

TABLE X. Leaf-wise puparia per square-inch on the basis of the affected area of the leaf as well as on the basis of 10 random inch-units with the 't'-values of the differences in each patch (or zone); Estimates of the variances and the error per cent. of the estimate of incidence—Plot (1), Plot (2)

Leaf No.	Low			Medium			Severe			Pooled			Low			Medium			Severe			Pooled		
	Pup. per sq. in. of leaf	Pup. per sq. in. on the basis of 10 units	Pup. per sq. in. of leaf	Pup. per sq. in. of leaf	Pup. per sq. in. from 10 units	Pup. per sq. in. of leaf	Pup. per sq. in. of leaf	Pup. per sq. in. from 10 units	Pup. per sq. in. of leaf	Pup. per sq. in. of leaf	Pup. per sq. in. from 10 units	Pup. per sq. in. of leaf	Pup. per sq. in. of leaf	Pup. per sq. in. from 10 units	Pup. per sq. in. of leaf	Pup. per sq. in. of leaf	Pup. per sq. in. from 10 units	Pup. per sq. in. of leaf	Pup. per sq. in. of leaf	Pup. per sq. in. from 10 units	Pup. per sq. in. of leaf	Pup. per sq. in. of leaf	Pup. per sq. in. from 10 units	Pup. per sq. in. of leaf
1	2.97	3.14	4.93	6.67	6.73	12.45	17.01	2.41	2.57	14.82	2.03	8.31	4.93	6.51	1.29	2.97	3.14	4.93	6.67	6.73	12.45	17.01	2.41	2.57
2	0.99	0.91	16.38	2.98	3.00	3.84	3.81	0.63	1.71	2.61	6.86	2.98	3.29	0.76	0.89	1.29	1.29	16.38	2.98	3.00	3.84	3.81	0.63	1.71
3	1.29	1.43	4.22	2.98	3.00	0.27	0.12	0.48	..	3.77	3.71	19.35	22.00	9.18	11.60	1.29	1.43	4.22	2.98	3.00	0.27	0.12	0.48	..
4	2.30	2.27	5.79	5.40	0.55	0.97	1.00	0.20	0.18	2.18	1.34	2.39	3.06	6.14	6.14	2.30	2.27	5.79	5.40	0.55	0.97	1.00	0.20	0.18
5	0.31	0.22	0.64	0.71	3.69	0.54	1.29	1.03	0.63	1.82	1.30	4.95	6.19	2.98	2.86	0.31	0.22	0.64	0.71	3.69	0.54	1.29	1.03	0.63
6	1.08	1.57	1.45	1.79	6.01	13.25	8.29	0.57	0.57	3.23	1.67	2.64	2.86	3.21	3.86	1.08	1.57	1.45	1.79	6.01	13.25	8.29	0.57	0.57
7	1.91	1.75	2.17	3.00	6.12	4.00	5.97	0.50	0.91	5.39	3.78	2.66	0.95	1.60	2.14	1.91	1.75	2.17	3.00	6.12	4.00	5.97	0.50	0.91
8	1.26	2.29	1.35	1.69	4.91	21.18	18.30	1.29	1.86	5.39	3.78	2.66	0.95	1.60	2.14	1.26	2.29	1.35	1.69	4.91	21.18	18.30	1.29	1.86
9	1.39	1.57	1.10	0.71	34.08	35.87	32.72	7.48	8.81	1.08	1.63	2.95	5.36	6.11	0.79	1.39	1.57	1.10	0.71	34.08	35.87	32.72	7.48	8.81
10	0.86	0.39	22.64	4.29	17.57	6.41	7.43	1.08	2.29	4.57	5.00	3.39	2.74	2.64	4.60	0.86	0.39	22.64	4.29	17.57	6.41	7.43	1.08	2.29
11	0.63	..	4.32	4.29	13.39	2.07	1.82	0.22	0.10	6.57	7.14	7.00	2.74	2.64	4.60	0.63	..	4.32	4.29	13.39	2.07	1.82	0.22	0.10
12	1.42	1.81	7.61	1.07	2.88	0.22	0.29	1.04	0.89	0.99	0.11	27.53	8.90	0.48	0.24	1.42	1.81	7.61	1.07	2.88	0.22	0.29	1.04	0.89
13	0.46	1.54	0.90	0.82	7.61	3.23	0.38	4.14	5.45	4.49	0.95	4.52	3.21	15.66	15.71	0.46	1.54	0.90	0.82	7.61	3.23	0.38	4.14	5.45
14	1.15	1.05	6.21	0.45	9.17	2.22	0.38	4.14	5.45	4.49	0.95	4.52	3.21	15.66	15.71	1.15	1.05	6.21	0.45	9.17	2.22	0.38	4.14	5.45
15	2.84	1.10	7.06	1.43	7.29	6.86	6.00	0.69	0.29	1.51	2.86	7.32	6.35	2.93	6.00	2.84	1.10	7.06	1.43	7.29	6.86	6.00	0.69	0.29
16	0.87	7.79	7.87	1.00	8.14	2.08	1.05	3.38	2.21	3.10	2.26	1.75	2.00	1.28	2.38	0.87	7.79	7.87	1.00	8.14	2.08	1.05	3.38	2.21
17	8.99	9.21	5.02	6.75	5.45	1.10	0.20	1.08	0.41	2.15	1.87	10.31	3.31	2.07	0.92	8.99	9.21	5.02	6.75	5.45	1.10	0.20	1.08	0.41
18	1.89	1.56	5.22	5.83	7.43	1.47	1.21	1.71	1.43	0.67	1.02	3.20	33.90	1.08	2.32	1.89	1.56	5.22	5.83	7.43	1.47	1.21	1.71	1.43
19	1.63	1.82	2.54	0.82	2.60	3.69	1.98	0.67	0.86	2.31	3.57	6.32	7.43	1.90	3.02	1.63	1.82	2.54	0.82	2.60	3.69	1.98	0.67	0.86
20	0.56	0.54	5.01	7.98	1.43	4.12	3.33	0.44	0.79	2.16	2.86	12.79	20.32	3.32	2.43	0.56	0.54	5.01	7.98	1.43	4.12	3.33	0.44	0.79
Total	34.80	50.14	174.46	54.96	179.06	122.84	115.37	30.43	33.39	86.64	59.32	136.74	146.51	93.85	102.45	34.80	50.14	174.46	54.96	179.06	122.84	115.37	30.43	33.39

$\bar{t}$	..	1.437 N.S.	1.82 N.S.	0.205 N.S.	0.883 N.S.	0.970 N.S.	0.512 N.S.	0.254 N.S.	0.876 N.S.
$E(\sigma_1^2)$	..	1.2712	2.7217	60.6402	38.3455	1.296	9.9252	35.6738	5.0888
$E(\sigma_2^2)$	..	2.0772	7.9630	48.6444	32.7656	1.513	8.5010	30.0040	12.3900
Error % of mean	21.59	21.27	26.28	32.77	11.42	26.97	27.08	20.40	20.40

reasonably near the true value leaf by leaf. But the error per cent. with only 20 leaves in the whole patch and 10 units per leaf is not sufficiently low as will be noticed in the last line of the table. If instead of 2 leaves per unit, as many as 20 leaves per unit be selected (as recommended before) and then 10 inch units (about 25 per cent. of the inch-units) from each leaf be chosen at random, the error per cent. will come down to near about 5 per cent.

But from practical point of view, the selection of 10 random inch-units in a leaf followed by the counting of the puparia unit by unit will probably be more difficult than counting the puparia of the entire leaf itself. Even if these two methods involve almost equal labour, enumeration of the whole leaf is preferred since complete enumeration of a leaf will avoid the possibility of the further error introduced by sampling.

## VII. SUMMARY OF CONCLUSIONS

(i) The incidence of white-fly is highly variable and the maximum variation occurs in leaves. The variations between the clumps and the canes if and when they exist are of a much lesser magnitude. Therefore, increase in the number of leaves alone in a sample is pre-eminently effective in the reduction of error of an estimate.

(ii) In a field where the infestation is either high or fairly high (puparia per square inch of the affected area more than 5.00), Nested sampling has to be adopted so as to ensure the equitable representation of clumps, canes and leaves in a sample. In such a field, 10-13 per cent. of the 3 ft. units should first be selected and then Nested sampling done in each unit in that particular form of the alternatives, which gives the larger number of leaves in the sample, namely 2 clumps  $\times$  2 canes  $\times$  5 leaves per unit or 20 affected leaves equitably representing the clumps and canes in a unit as far as possible or 18-20 per cent. of affected leaves (*i.e.*, about one in every five) equitably representing the clumps and canes in a unit.

(iii) In a plot of low or mild infestation, Nested sampling is not essential. Only the leaves as a whole may be subjected to random selection, other requirements being the same as mentioned under number (ii) above.

(iv) For a required error percentage, a medium infested field (puparia, 2.00-5.51 per square inch) requires a smaller sample size than that needed in a plot of high infestation or of an infestation which is fairly high. A difference in the sample size in the number of leaves by even about 75% gave the same efficiency. This suggested inherent homogeneity in variation occurring in mild form of infestation.



(v) If in each leaf, 10 random inch units (about 25% of the total inch units) be taken instead of enumerating the whole leaf, the estimate of the incidence will be fairly reliable with a very slight additional error; but for practical consideration and working convenience, the complete enumeration of a leaf may be preferred to counting puparia in 10 random inch-units.

### VIII. ACKNOWLEDGMENTS

The work was carried out as part of the Sugarcane Research Scheme in Bihar being financed jointly by the Bihar Government and the Imperial Council of Agricultural Research, to whom grateful thanks are due. Sincere appreciation of the facilities afforded to field staff by the management of the Rohtas Industries Ltd., Dalmianagar in whose area the field study was made, is also recorded. Similarly assistance rendered in field collection and population counts by Mr. A. C. Sen, Senior Entomological Assistant, is acknowledged.

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# STUDIES ON THE REFRACTIVE INDEX OF MILK

## III. Detection of Adulteration in Milk

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THE first two papers on refraction in milk (Rangappa, 1947) dealt with the limits of the refractive index and refractive constant of genuine cow and buffalo milk under natural conditions of variation. In this paper the applicability of the two values for detection of adulteration of milk has been investigated.

Adulteration of milk is practised in various ways. But the most common of these is dilution with water. The practice is so rampant to-day and so little attention is being paid towards its prevention that genuine milk is hard to come by even at unreasonably high prices in most of the cities in India.

In places where authorities are somewhat vigilant more subtle forms of sophistication come into play. Milk is then either partially defatted, or skim milk is added to whole milk. Where tests for adulteration are not sufficiently refined, water is added to the mixture of whole and skim milk in quantities sufficient to restore the raised density to normal levels.

The crudest form of adulteration, next only to addition of mere water, is addition of sugar or jaggery (crude or country sugar) to watered milk to the extent of raising the density to average levels.

Other and more easily detectable forms of adulteration of milk are admixture of watered milk with fine flour to mask the thinness. In such cases sedimentation or test with iodine at once reveals the presence of starch.

In this part the results of experiments on the detection of added water, skim milk or sucrose to watered milk by measuring the refraction in the sample are described. The application, utility and limitations of this method for each type of adulteration are dealt with separately and compared with current methods of detection.

## DETECTION OF ADDED WATER

The methods now in vogue for detection of adulteration with water are chiefly the standards of fat and fat-free solids in milk, and the depression of the freezing point.

The presence of two different species of milch animals in India has involved the institution of separate standards for each. Thus for the Indian cow a minimum of 3.5 per cent. fat and 8.5 per cent. solids-not-fat, and for the buffalo 5 per cent. fat and 8.5 per cent. solids-not-fat are fixed in some Provinces. It is now recognised and has also been indicated by the author (*cf.* Part I) that these minima appear to stand in need of revision in the light of the data obtained on the composition of milk in different parts of the year.

Elsdon and Stubbs (1929) have carried out numerous determinations of refractive index of milk-serum. Leather (1930-31), Bunce (1932) and Macmahon and Srivastav (1935) have determined the freezing point of milk of the Indian cow and buffalo. From these data it is seen that the range of the constant extends from  $-0.518^{\circ}$  to  $-0.580^{\circ}$  C. for cow, and from  $-0.521^{\circ}$  C. to  $-0.590^{\circ}$  C. for buffalo milk. It follows therefore that in spite of the sensitiveness of the test a minimum of 3-5 per cent. dilution with water is possible. Fixing the normal minimum of freezing point of cow milk as  $-0.530^{\circ}$  C. a dilution of 5-6 per cent. is possible, and with buffalo milk a still higher percentage is possible. And the claim of Bunce that the freezing point can serve as a distinguishing test is, in view of the large overlapping of the ranges of the constant of the two milks, manifestly indefensible.

Ghosh and Datta Roy (1941) have suggested that the lactose-fat ratio in milk could be utilised not only for detecting added water but to classify the sample under examination as cow or buffalo milk. But this naturally requires the estimation of both fat and lactose, which is obviously impracticable in a public analyst's laboratory where numerous samples come in for quick disposal.

The determination of the refractive index and the lactometer reading of milk, which is, as simple and quick as it is accurate, can be pushed into service as an efficient weapon in the detection of adulteration.

## EXPERIMENTAL PROCEDURE

Genuine samples of cow and buffalo milk of different grades of refractive index and refractive constant, with values mostly above the average, were systematically watered and the changes in the two constants observed at every step. Table I and Fig. 1 give the results of the experiments.

TABLE I. *Change of Composition and Refractive Index and Refractive Constant with Systematic Addition of Water to Cow and Buffalo Milk*

Added Water %	Density (20°C.)	Fat %	Total solids % (calculated)	S. N. F. %	R. I. (40°C.)	K
COW						
Sample I						
0	1.0294	4.0	13.02	9.02	1.3462	0.2074
15	254	3.5	11.24	7.74	38	64
30	214	3.0	9.64	6.64	23	64
Sample II						
0	1.0284	5.0	13.81	8.81	1.3461	0.2071
15	250	4.2	11.99	7.79	37	63
30	221	3.5	10.42	6.92	22	62
50	182	3.3	9.20	5.90	08	63
Sample III						
0	1.0256	..	..	..	1.3449	0.2066
10	240	..	..	..	28	63
25	214	..	..	..	20	60
Sample IV						
0	1.0278	..	..	..	1.3458	0.2069
10	247	..	..	..	40	66
25	223	..	..	..	23	62
50	186	..	..	..	07	62
Sample V						
0	1.0270	..	..	..	1.3456	0.2068
10	249	..	..	..	36	65
25	219	..	..	..	24	62
50	184	..	..	..	11	61
BUFFALO						
Sample I						
0	1.0289	6.6	15.87	9.27	1.3480	0.2083
15	251	5.8	13.95	8.15	48	71
30	223	5.1	10.49	6.39	34	69
Sample II						
0	1.0284	6.6	15.75	9.15	1.3474	0.2085
10	254	..	..	..	54	72
25	222	5.2	12.39	7.19	34	70
50	..	4.5	..	..	20	..
Sample III						
0	1.0319	7.5	17.71	10.21	1.3491	0.2081
10	277	6.8	15.81	9.01	70	77
20	..	6.25	14.65	8.40	56	..
25	244	6.0	14.02	8.02	51	73
50	208	5.0	11.91	6.91	32	71
Sample IV						
0	1.0302	7.6	..	..	1.3487	0.2082
10	273	..	..	..	59	71
25	241	..	..	..	39	67
50	..	..	..	..	22	..
Sample V						
0	1.0303	7.2	..	..	1.3482	0.2031
10	273	..	..	..	58	70
30	229	..	..	..	35	68
Sample VI						
0	1.0282	..	..	..	1.3486	0.2086
10	254	..	..	..	64	79
25	225	..	..	..	44	74

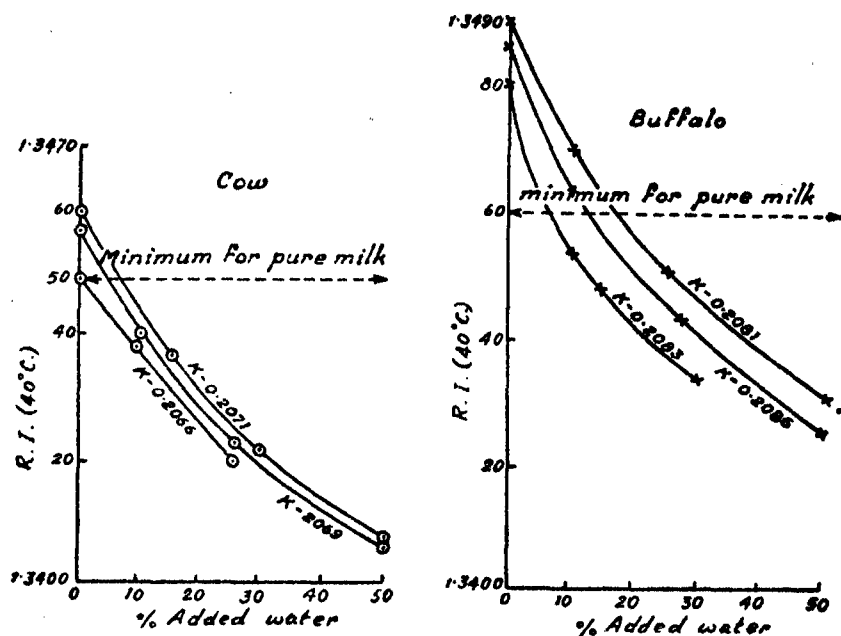


FIG. 1. Fall of R. I. of Milk on Dilution with Water.

Now, taking into consideration the refractive index alone, it will be seen from the above table and the accompanying figure, that added water reveals itself in samples of cow milk of very high refractive index (1.3470\* and over) generally at a maximum of 10 per cent. and more of addition. But as indicated in Part I, the most commonly occurring values are 1.3461 and those near it. Therefore with average and bulk samples, adulteration with water begins to reveal itself at levels of less than 8 per cent. addition. Thus with a sample of refractive index 1.3454 (*cf.* figure) hardly 3 per cent. water can be added without lowering the constant below the minimum value for milk.

Samples of buffalo milk with very high refractive indices, (1.3491) on the other hand, allow of about 20 per cent. addition of water. At this level of dilution, it will be seen from the table, the rich sample with refractive index 1.3491 will pass off, on the basis of chemical composition, as genuine, since it conforms to the presumptive standards. But the lowered refractive index (1.3456 at 20% added water) definitely indicates adulteration even when the composition is supposedly normal. In other words, the refractive index indicates dilution even before the composition is lowered below the presumptive standards for fat and solids-not-fat of buffalo milk. With

\* All values of refractive index are given at 40° C.

average samples (mode, 1.3480) dilution is detected at levels of about 10 per cent. addition. The figure shows (by extrapolation) that an average sample with refractive index 1.3468 does not allow of more than 5 per cent. of added water.

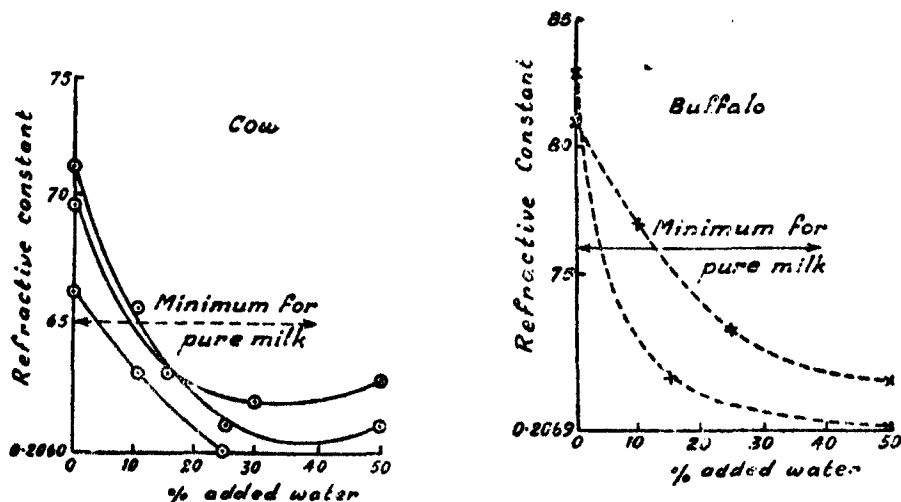


FIG. 2. Fall of Refractive Constant of Milk on Dilution with Water

○—○ Cow milk  
x—x Buffalo milk

On the basis of refractive index alone, however, it is clearly possible to tone down buffalo milk and pass it off as cow milk. Samples of buffalo milk with very high refractive index can thus be diluted to the extent of about 25 per cent., and with normal values to about 15 per cent. of added water. And it is here that the refractive constant comes in as a handy weapon. Further, judgment only on the basis of refractive index suffers from the disadvantage, pointed out by Elsdon and Stubbs (1929) that genuine samples of milk with very low solids-not-fat also possess values of refractive index below the minimum for normal samples, so much so, samples clearly diluted have to be given the benefit of doubt on the basis of this observation. This limitation is at once overcome by taking into account the refractive constant also of the sample, which has already been shown (Part II) to remain within narrow limits in spite of variations in milk composition.

Comparing Figs. 1 and 2 it will be seen that in the case of the samples of milk with refractive index much above the average, the refractive constant clearly narrows down the margin of adulteration. Thus with the buffalo milk sample of refractive index 1.3491, the allowance for dilution

of 20 per cent. is reduced to 12-15 per cent. when the refractive constant is taken into consideration. Similarly with the sample of refractive index 1.3480, the allowance of adulteration of 8 per cent. is reduced, on the basis of the refractive constant to about 5 per cent.

With samples of average and less-than-average refractive index, it follows, the margin of adulteration is equally small both on the basis of refractive index as well as of the constant.

Attention may also be drawn to the fact that the lack of correlation between the refractive index and constant of milk (Parts I and II) has a certain advantage in the problem of detection of adulteration; for any given value of refractive index, the corresponding value of refractive constant varies over a wide range. As often as not a high refractive index is associated with comparatively low values of refractive constant, and *vice versa*. Therefore, while one may allow a comparatively wide margin for dilution, the other will narrow it down to a minimum.

The preceding table indicates also the method of detection of diluted buffalo milk that is designed to pass off as cow milk. It will be observed that dilution of buffalo milk promptly disturbs the relationship between the refractive index and constant. In sample I (buffalo) for instance, at 15% dilution although the refractive index is below the minimum for genuine cow milk, the refractive constant is quite within the range for cow milk, but too low for normal buffalo milk. In other words, had the sample belonged to cow the refractive constant also would ordinarily have been, at that dilution (or for that value of R.I.), below the minimum for cow milk (0.2065). Similarly the values after further dilution in this and other samples indicate how this type of fraud can also be uncovered. That is, values of refractive index *below* the normal coupled with refractive constant *within* the normal range for cow milk indicate watered buffalo milk.

The preceding discussion stresses, therefore, the necessity and importance of viewing both the refractive index and refractive constant of milk in conjunction with each other.

#### RATE OF VARIATION OF REFRACTIVE INDEX AND REFRACTIVE CONSTANT OF MILK ON DILUTION WITH WATER

Figs. 1 and 2 furnish further valuable information. It will be observed that the rate of fall of refractive index remains the same in all cases, depending as it does on the steady fall in concentration of the fat-free solids of milk, of which refractive index is a function. The refractive constant, on the other hand, falls at variable rates on dilution. This is accounted for by the fact that the refractive constant involves both the refractive index

and the density, and that, although the former varies in a regular fashion, the density changes at different rates with dilution depending on its initial fat content. To explain more fully, any given value of refractive index may be found associated with several different values of density owing to the variable fat content in milk samples. Therefore samples with the same refractive constant could result from combinations of refractive index and density of different magnitudes; and again, a given density can be the result of different combinations of fat and fat-free solids of milk. It is the latter fact that is responsible for different rates of fall of density with dilution, which in its turn, renders the fall of the refractive constant variable. Fig. 3 illustrates this fact clearly.

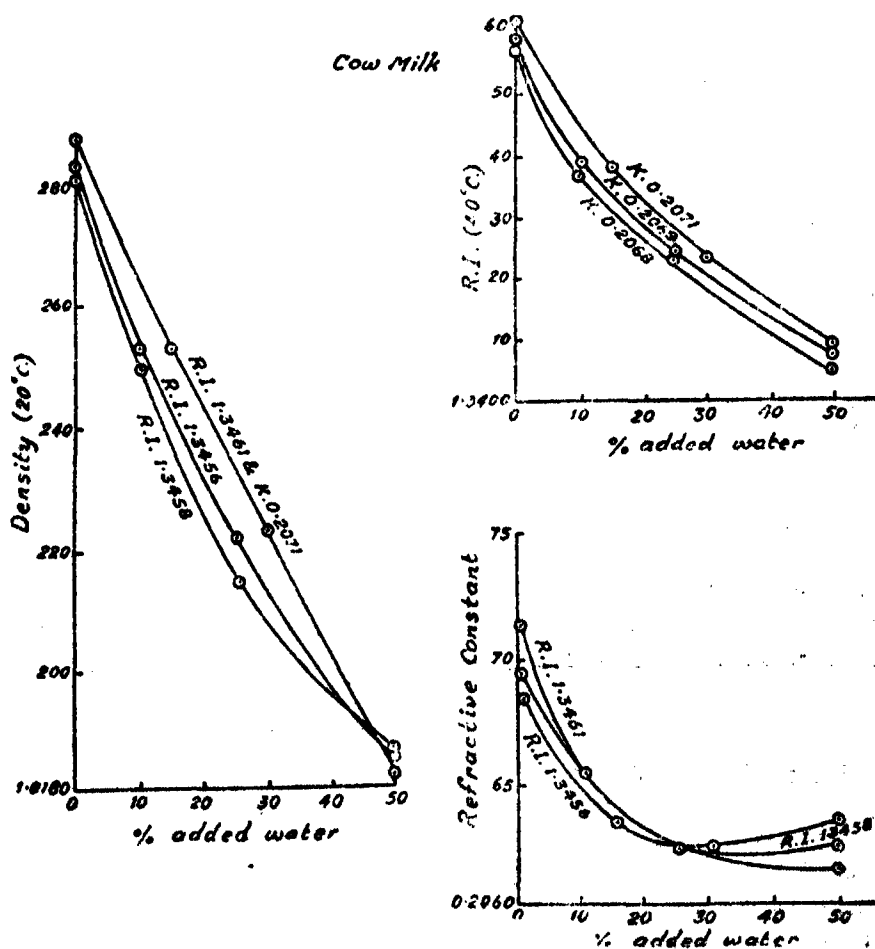


FIG. 3. Variation of Refractive Index, Constant and Density of Milk on Dilution with Water



## DETECTION OF ADULTERATION WITH SKIMMED MILK

Watering milk does not in general change the relation of the various constituents to one another since these are all reduced in the same proportion. On the other hand if skim milk is added to or fat is removed from whole milk the ratio of fat to the other constituents is promptly altered. This fact has been utilised in the tests now in official use for defatting and/or added skim milk.

In general it has been fixed that the protein: fat ratio should never exceed 1.0, as the fat content of a sample has always been found to exceed its protein content (Lythgoe, 1910). But the ratio of 1.0 is too generous a standard, and, in judging the purity of a sample, should not be taken too literally as a criterion. Lythgoe (1921) has found that the mixed milk of the Guersny and Jersey cows has a protein-fat ratio as low as 0.6. It will thus be seen that the margin for adulteration, with this standard, is indeed very wide. And again, every test involves the determination of both fat and nitrogen content of the sample.

The specific gravity of milk solids has also been used as a criterion for detecting added skim milk, or of defatting. Calculated on the basis of Fleischmann's formula, the specific gravity of normal milk solids has been found to vary between 1.25 and 1.34. While this is not changed by watering, defatting or addition of skim milk raises the specific gravity of the solids. A value of 1.4 is taken as conclusive evidence of skimming or of addition of skim milk. Therefore this standard also allows of liberal adulteration in this respect.

Taking the refractive index and refractive constant into consideration it will be observed that while the refractive index remains unaltered by defatting or by addition of skim milk, the refractive constant steadily decreases owing to the rising density. The rate of change of the constant with addition of skim milk is given in the following experiment.

## EXPERIMENTAL PROCEDURE

From a 2 litre lot of genuine milk of known refractive index one litre was skimmed in a hand-worked separator leaving hardly 0.2 per cent. of fat in the skim milk. The latter was added in increasing percentages to the whole milk and density noted at every addition. The fall of the refractive constant in the process was then estimated. The experiment was repeated with samples of buffalo milk also. Table II and Fig. 4 indicate the trend of variation of the refractive constant with this type of adulteration.

TABLE II. *Change of Composition and Refractive Constant of Cow and Buffalo Milk with Systematic Addition of Skim Milk*

Added skim milk %	Density (20° C.)	Fat %	Total Solids % (calculated)	S. N. F. %	K
COW MILK					
Sample I (R. I. 1.3462)					
0 ..	1.0234	5.2	14.05	8.85	0.2072
10 ..	292	4.7	13.65	8.95	70
25 ..	296	4.15	13.08	8.93	69
50 ..	305	3.5	12.52	9.02	67
Skim milk ..	342	0.2	9.45	9.25	60
Sample II (R. I. 1.3480)					
0 ..	1.0299	5.1	..	..	0.2086
15 ..	305	..	..	..	63
30 ..	310	..	..	..	65
50 ..	316	..	..	..	64
Skim milk ..	352	..	..	..	56
BUFFALO MILK					
Sample I (R. I. 1.3492)					
0 ..	1.0292	7.8	17.40	9.80	0.2085
10 ..	299	7.1	16.73	9.63	84
25 ..	308	6.2	15.86	9.66	83
50 ..	324	5.2	15.05	9.85	79
Sample II (R. I. 1.3470)					
0 ..	1.0264	..	..	..	0.2050
25 ..	292	..	..	..	74
50 ..	299	..	..	..	72
100 ..	312	..	..	..	69
Skim milk ..	354	0.25	..	..	62
Sample III (R. I. 1.3477)					
0 ..	1.0291	6.9	..	..	0.2079
10 ..	311	..	..	..	77
25 ..	323	..	..	..	74
50 ..	331	..	..	..	73
75 ..	340	..	..	..	70
100 ..	349	..	..	..	68
Skim milk ..	379	0.2	..	..	63
Sample IV (R. I. 1.3500)					
0 ..	1.0303	9.3	..	..	0.2088
15 ..	318	..	..	..	85
30 ..	328	..	..	..	84
50 ..	339	..	..	..	81
Skim milk ..	410	0.2	..	..	67

It is clear from the figure and the above table that the sensitivity of the test is not the same for all samples; for, the rate of fall of refractive constant with addition of skim milk or of defatting is, unlike in the case of added water, very slow with the consequence that samples with very high values of refractive constant allow of very large (over 50 per cent.) dilution with skim milk. But the test is not, it will be observed, altogether useless. The most frequently occurring values of refractive constant, especially of buffalo milk, being much below the maximum value, added skim milk is normally detected at levels of 15 to 20 per cent.

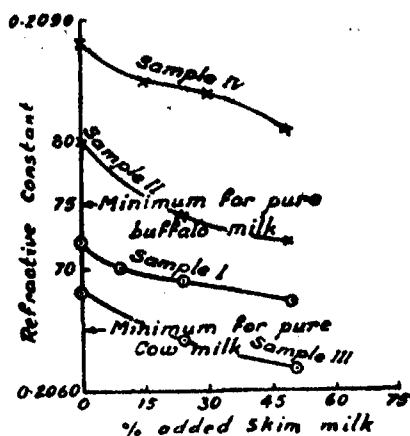


FIG. 4. Changes in Refractive Constant of Milk on Dilution with Skim Milk

○—○ Cow milk  
 ×—× Buffalo milk

Attempts may however be made to prevent the rise in density, caused by defatting or by addition of skim milk, by addition of water. But this procedure naturally lowers the refractive index which is at once detected—one and the same test thus preventing both methods of sophistication.

#### EFFECT OF ADDED SUCROSE TO WATERED MILK ON REFRACTIVE INDEX AND REFRACTIVE CONSTANT OF MILK

One of the more common frauds practised by milk vendors who are wise to the detection of added water in milk by lowered density is to add sugar to watered milk. This, unlike added flour, leaves no sediment but raises the lactometer reading. In food analysis a separate test is resorted to for detecting added sucrose, large additions being easily detected by the unnatural sweetness of the product. Now, with the advent of the method of determining refractive index, the test has been attempted to be pressed into service for uncovering this type of adulteration as well.

#### EXPERIMENTAL PROCEDURE

Normal samples of cow and buffalo milk were systematically watered, enough sugar added to restore the density to the original level and the change of refractive index and constant caused by this procedure observed. The resulting data are given in Table III and graphically represented in Fig. 5.

TABLE III. Effect of Addition of Sucrose to Watered Milk

Sample	Density (20° C.)	Fat %	Total Solids % (calculated)	S. N. F. %	R. I. (40° C.)	K
Cow						
Sample I						
(1) Genuine milk ..	1.0294	4.0	13.02	9.02	1.3462	0.2074
(2) + 15% water ..	254	3.5	11.24	7.74	38	64
(3) + Sucrose ..	290	3.5	..	..	48	63
(4) + 30% water ..	214	3.0	9.64	6.64	23	64
(5) + Sucrose ..	293	..	..	..	38	56
Sample II						
(1) Genuine milk ..	1.0274	5.0	13.81	8.81	1.3461	0.2071
(2) + 15% water ..	250	4.3	12.11	7.81	37	63
(3) + Sucrose ..	281	..	..	..	46	64
(4) + 50% water ..	182	3.3	9.20	5.90	08	63
(5) + Sucrose ..	279	..	..	..	37	58
BUFFALO						
Sample I						
(1) Genuine milk ..	1.0289	6.6	15.87	9.27	1.3480	0.2083
(2) + 15% water ..	251	..	..	..	48	71
(3) + Sucrose ..	286	..	..	..	65	73
(4) + 30% water ..	223	5.1	12.41	7.31	34	69
(5) + Sucrose ..	284	..	..	..	60	60

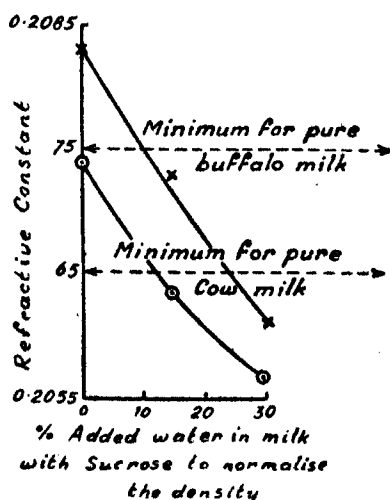
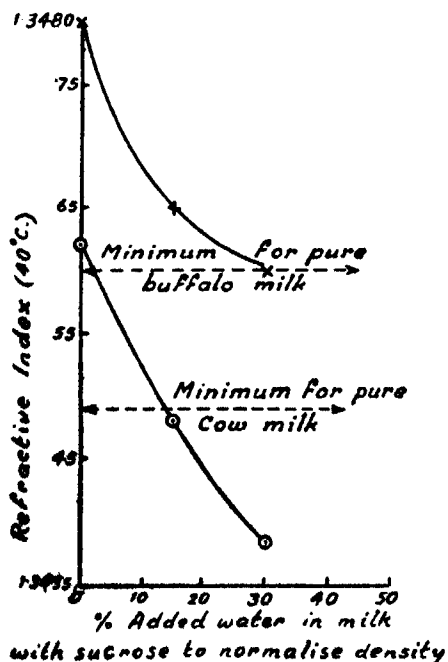


FIG. 5a. Changes in R. I. when Sucrose is added to Watered Milk to Restore the Density.  
 5b. Changes in Refractive Constant when Sucrose is added to Watered Milk to Restore the Density.

○—○ Cow milk  
 ✕—✕ Buffalo milk

It is clear from the table and figure that the attempt to restore the density of watered milk by adding sugar fails to raise the refractive index to its original value. But with average samples this procedure helps to raise the refractive index above the minimum for genuine milk (though not to its original level) up to about 12–15 per cent. of added water in case of cow, and about 30 per cent. of added water with buffalo milk. On the other hand, the artificially raised density and the lowered refractive index (below the original value) promptly contribute towards a reduction of the value of the refractive constant below the minimum for pure milk. Thus, taking this constant into consideration, the margin of dilution in average samples is reduced to about 10 per cent. in cow and to 15 per cent. in buffalo milk. Further, attempts to raise the refractive index to normal or higher levels by further additions of sugar raise the density still more, and therefore only succeed in reducing the value of the refractive constant to levels still further below the minimum and also make the sample unnaturally sweet.

#### SUMMARY

The data collected and the limits of refractive index and constant of genuine milk worked out in the last two papers have been utilised in the attempt to detect different types of adulteration.

Samples of milk of different grades of refractive index and constant have been systematically watered and their rate of fall of the values determined. By viewing the two determinations in conjunction with each other it has been found possible to detect in average samples dilution with water up to a minimum of about 5–8 per cent.

Watered buffalo milk designed to pass off as cow milk reveals itself by the disturbed relationship between the refractive index and constant. While the refractive index of such a sample is normal for cow milk the refractive constant will be found to be usually too high for that value of the refractive index.

Added skim milk or removal of fat is detected by the lowered values of refractive constant, which in average samples is reduced below the minimum for pure milk at about a minimum of 20–25 per cent. addition. With samples of very high refractive constant, however, considerably larger dilutions (up to 50 per cent.) are possible.

Attempts at normalising the density of watered milk by adding sugar are detected up to about 10 per cent. added water in cow, and about 12–15 per cent. in case of buffalo milk samples.

Thus a simple determination of the refractive index and density of milk serves to detect a number of common types of adulteration of this product.

### CONCLUSIONS ON THE REFRACTIVE INDEX OF MILK

The investigations so far described (Parts I to III) have brought out the simplicity, degree of precision and the wide applicability of the estimation of Refractive Index of Milk.

The advantages of the method can be summarised as follows:—

The ease of determination and the accuracy of the estimation are well known. It requires hardly 30 minutes to test a dozen samples by the method described. Unlike in the case of the Freezing Point test, this method can be carried out by any trained laboratory assistant without much claim to skilled technique. The Abbe' refractometer is much less delicate, less expensive and far more easily handled and cared for than the Hortvet apparatus. Considering the advantages of the method it is quite sensitive and accurate, and under practical conditions reduces the margin of adulteration to a very low minimum. The method offers the simplest procedure of distinguishing between cow and buffalo milk.

Finally, it serves as a multipurpose test—an advantage possessed by no other single test inasmuch as a simple estimation of refractive index and density uncovers adulteration with water and sugar in watered milk.

### ACKNOWLEDGMENT

I am thankful to Prof. V. Subrahmanyam and Mr. B. N. Banerjee for their kind interest and encouragement in all these investigations.

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# A CONTRIBUTION TO THE EMBRYOLOGY OF *ACACIA FARNESIANA* L. (WILLD.)

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## INTRODUCTION

THE literature on the Leguminosæ has been reviewed by Maheshwari (1931) and later by Roy (1933) and Newman (1934). The earliest important work on the embryology of the family is that of Guignard (1881) covering about forty species of the family wherein some details of the reproductive organs and embryology are given. In the sub-order Mimosaceæ, Guignard (1881) has investigated some of the species of *Acacia*, while Maheshwari (1931) has worked on the morphology of *Albizzia Lebbek*. The latest detailed paper on Mimosaceæ is that by Newman (1934) on *Acacia Baileyana*.

The present paper deals with the study of endosperm formation and embryo development in *Acacia farnesiana*, L. (Willd.). The plant flowers in all seasons. The material was fixed in Allen's modified Bouin. Sections were cut from 8–12  $\mu$  in thickness and stained in Heidenhain's iron-alum-hæmatoxylin.

## MICROSPORANGIUM

The wall of the young anther (Fig. 1) shows beneath the epidermis, a prominent endothecium which develops fibrous thickenings at maturity, two middle layers and the tapetum. The tapetum remains uninucleate throughout as observed by Maheshwari (1931) in *Albizzia Lebbek*. The microspore-mother-cells after undergoing the reduction divisions produce 8 or 16 microspores in each sporangium. The individual microspores do not separate, but are held together as a unit and are shed as a single mass. At the time of shedding (Fig. 2) each pollen grain contains a generative and a tube nucleus. The same condition is observed in *Albizzia Lebbek* and *Acacia Baileyana* by Maheshwari (1931) and Newman (1934) respectively.

## MEGASPORANGIUM

As is characteristic in the Leguminosæ, the ovules are arranged in two rows on the marginal placenta of the monocarpellary ovary. The number

may vary from 6-14. The ovule arises as a blunt papillate process and consists at first of a group of homogeneous cells. The archesporium is hypodermal in origin and consists of a single cell (Fig. 3). Sometimes the archesporium consists of two (Fig. 4) or more cells, but usually only one cell develops further. The archesporium cuts off one or two parietal cells which may divide further both anticleinally and pericleinally forming a wall of 4-5 layers in thickness.

The megaspore-mother-cell during the course of further development crushes the surrounding nucellar cells and enlarges in size. It undergoes the usual meiotic divisions (Figs. 5 and 6) and a linear tetrad is formed (Fig. 7). Occasionally a T-shaped tetrad is also met with (Fig. 8). In either case the upper three megaspores degenerate, and the lowermost develops into the normal monosporic 8-nucleate embryo-sac (Figs. 9 and 10).

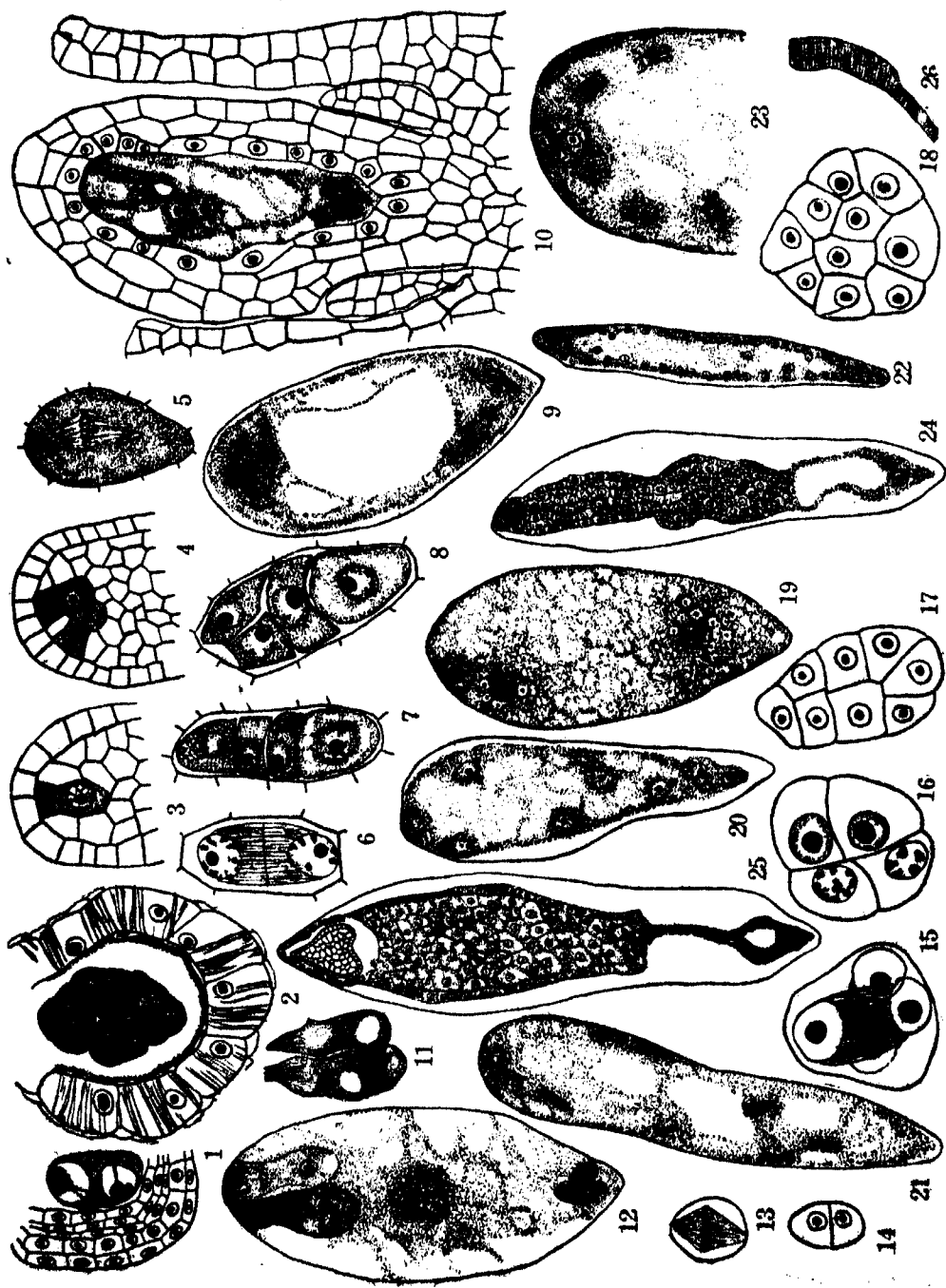
The mature embryo-sac is broad at the micropylar end and narrow at chalazal end. The enlargement of the embryo-sac does not obliterate all the nucellar tissues at the time of fertilization as recorded by Guignard (1881), but some portion is left at the sides near the chalazal region as observed by Newman (1934) in *Acacia Baileyana*.

The integuments are rather belated in appearance and though the inner integument appears first, the outer integument soon outgrows the inner one. When the mature embryo-sac is organised, the inner integument covers only the lower half of the embryo-sac while the outer integument is in level with the apex of the nucellus. Guignard (1881) records that both the integuments are in level with the apex of the nucellus at the time of the maturity of the embryosac, and Newman (1934) states that in *Acacia Baileyana* the ovule gets full integuments after fertilization, forming a micropyle by the time of the first division of the fertilised egg. The inner integument is only two cells in thickness at the micropylar region, the outer integument becomes massive especially at the top in the more advanced ovules.

The synergids are pear-shaped and slightly hooked (Fig. 11), possessing the characteristic filiform apparatus. A prominent vacuole is found below the nucleus. The position of the egg is variable, either below the synergids or away from the synergids, with a prominent vacuole above and the nucleus below. The antipodals are definite cells and persist for a long time beginning to degenerate only after fertilization. The two polars remain in contact without fusing till fertilization.

Varying amounts of starch grains are found in the embryo-sac from the 8-nucleate stage onwards upto the free nuclear condition of the endosperm. Guignard (1881) records starch grains in functional megaspores of *Acacia*





FIGS. 1-26

**FIGS. 1-26.** Fig. 1. Portion of a transverse section of young anther showing the epidermis, wall layers, tapetum and microspore mother cells  $\times 1,080$ . Fig. 2. Mature pollen grains remaining together at shedding condition, each having a generative and a tube nucleus  $\times 1,080$ . Fig. 3. Archeporsial cell with wall cell cut off  $\times 1,800$ . Fig. 4. Two archeporsial cells with parietal cells  $\times 1800$ . Fig. 5. Megaspore mother cell in metaphase  $\times 1,800$ . Fig. 6. Diad  $\times 1800$ . Fig. 7. Linear tetrad  $\times 1,800$ . Fig. 8. T-shaped tetrad  $\times 1,800$ . Fig. 9. Division of four nucleate into the eight nucleate embryo-sac  $\times 1,800$ . Fig. 10. Fully organised embryo-sac showing the egg apparatus, polar nuclei in contact and antipodals and the disposition of outer and inner integuments  $\times 1,440$ . Fig. 11. Details of the egg apparatus showing the filiform apparatus in the Synergids  $\times 1,800$ . Fig. 12. A stage in double fertilization showing the male nuclei in contact with the egg and one of the polars, antipodals still persisting  $\times 1,080$ . Fig. 13. First division of the fertilised egg  $\times 1,800$ . Fig. 14. Two-celled embryo  $\times 1,800$ . Fig. 15. Two-celled embryo dividing to form 4 cells.  $\times 1,800$ . Fig. 16. Four-celled embryo  $\times 1,800$ . Figs. 17-18. Eight and ten-celled embryos respectively  $\times 1,800$ . Fig. 19. Fertilized egg with two endosperm nuclei  $\times 1,440$ . Fig. 20. Fertilized egg with eight endosperm nuclei  $\times 1440$ . Fig. 21. Fertilised egg still undivided and endosperm nuclei in division  $\times 800$ . Fig. 22. Two-celled embryo with 64 endosperm nuclei  $\times 800$ . Fig. 23. Four-celled embryo and wall formation in the endosperm  $\times 1,800$ . Fig. 24. Endosperm with walls filling only the upper portion of the embryo-sac and lower portion with free endosperm nuclei  $\times 480$ . Fig. 25. Late embryo and lower portion of the embryo-sac having free endosperm nuclei  $\times 480$ . Fig. 26. Portion of seed showing the outermost layer of columnar cells  $\times 40$ .

*farnesiana* and Newman (1934) in *Acacia Baileyana*. According to Maheshwari (1931), however, starch grains are not seen before the 8-nucleate stage in *Albizia Lebbek*.

During fertilization, both syngamy and triple fusion (Fig. 12) occur normally. Remains of the pollen tubes may be found at the micropylar end even during the formation of free endosperm nuclei and occasionally two to three pollen tubes have been observed in the same ovule (Fig. 30) as in *Acacia Baileyana* (Newman, 1934). The further development of these could not be traced.

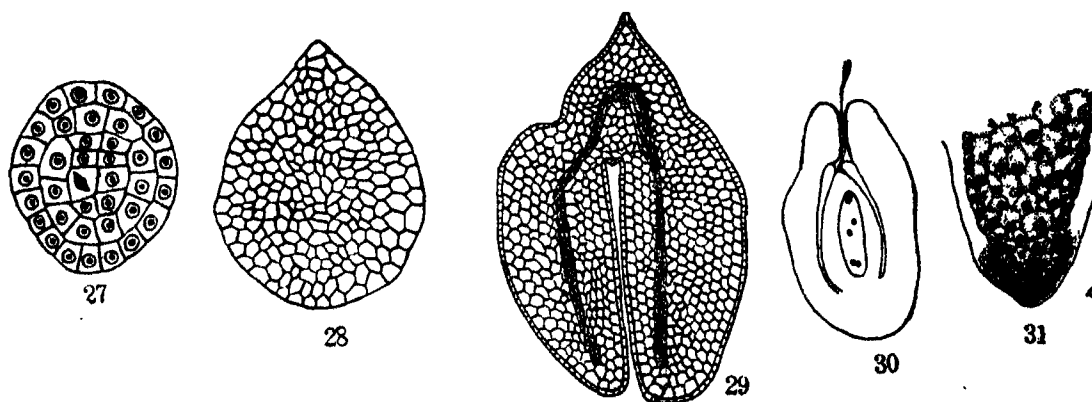
#### ENDOSPERM

The primary endosperm nucleus divides in a free nuclear manner, earlier than the fertilized egg. The zygote does not divide until 32 endosperm nuclei are formed and these latter are aggregated densely around the zygote and the chalazal region. Newman (1934) states that in *Acacia Baileyana* the zygote does not divide before the 8-nucleate stage of the endosperm as is also reported by Maheshwari (1931) in *Albizia Lebbek*. Further divisions continue until the whole embryo-sac is filled with endosperm nuclei (Figs. 19 to 22). Cell formation in the endosperm commences from the micropylar region at the four-celled stage of the embryo (Fig. 23), and extends only to the upper two-thirds of the embryo-sac, the chalazal end being filled with free endosperm nuclei. This condition persists even as late a stage as the appearance of the cotyledons in the embryo (Figs. 24, 25 and 31).

Newman (1934) finds that in *Acacia Baileyana* cell formation takes place at the 64 nucleate stage when the embryo is 12 celled. In the mature seed, all the endosperm is absorbed by the growing embryo.

### EMBRYO

The first division of the zygote is by a transverse (Figs. 13 and 14) wall which divides it into an upper and a lower cell. The second division is by a vertical wall wherein both the cells divide simultaneously (Figs. 15 and 16). Later divisions are irregular and soon the embryo assumes a pear-shaped body. There is no differentiation of a suspensor (Figs. 17, 18, 27 and 28) and the embryo is of a massive type. This conforms to the types of embryo



FIGS. 27-31. Figs. 27-28. Late stages of embryo  $\times 800$ . Fig. 29. Dicotyledonous embryo  $\times 40$ . Fig. 30. Two to three pollen tubes entering a single ovule  $\times 480$ . Fig. 31. Cellular endosperm and lower portion of the seed containing free endosperm nuclei  $\times 40$ .

in other species of Mimosaceae described by Guignard (1881) and Newman (1934). The mature embryo possesses a broad radicle with the vascular bundle on the adaxial face of the cotyledon (Fig. 29). The mature seed has a hard coat, the outermost cells of which are columnar (Fig. 26).

### SUMMARY

1. Pollen grains are shed united together in a mass of 8 or 16 from each sporangium. Each pollen grain possesses a generative and a tube nucleus at the shedding stage. The tapetum remains uninucleate throughout.

2. The archesporium may be single-celled or occasionally multicellular. Some amount of parietal tissue is formed by the division of the wall cells,

3. Ovules have two integuments which are belated in appearance. Both linear and T-shaped tetrads are formed, and the lowermost megaspore functions.

4. The embryo-sac conforms to the monosporic eight-nucleate type. Synergids show the characteristic filiform apparatus. Polar nuclei fuse after fertilization. Antipodals are definite cells and persist till fertilization. Double fertilization occurs normally.

5. Endosperm is free nuclear in the beginning and wall formation commences from the micropylar end, stopping short of the lower one-third of the embryo-sac. The chalazal end contains only free endosperm nuclei for a long period.

6. The first division of the fertilised egg is transverse and the second division is vertical. Later divisions are irregular. The embryo has no suspensor and is of the massive type.

In conclusion, grateful acknowledgments are made to Dr. L. S. Dorasami, M.Sc. (Lond.), Ph.D. (Lond.), Economic Botanist and Professor of Botany, for suggesting the problem and encouragement during the course of the work.

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# STUDIES ON THE ANATOMY OF THE TAIL IN SAURIA AND RHYNCHOCEPHALIA \*

## II. *Chameleon zeylanicus* Laurenti

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### I. INTRODUCTION

AN examination of the numerous papers published on the tail of lizards during the last two centuries shows that the only type which has attracted sufficient attention is the autotomous one. Müller (1852), Hyrtl (1853), Gegenbaur (1862), Fraisse (1885), Boulenger (1888), Werner (1892 and 1896), Tornier (1897), Misuri (1910), Woodland (1920), Slotopolsky (1921), White (1925), and Sibtain (1938) have all been interested in the morphological features of the tail associated with the act of autotomy; while the anatomy of the regenerated tail has been studied in more or less detail by Brindley (1894 and 1898), Morgan (1901), Annandale (1904), Woodland (1920), Terni (1922), Guyenot (1928), Marcucci (1932), Sibtain (1938), etc. Several authors (Brindley, 1894 and 1898; Fischer, 1907; Stuart, 1908; Gräper, 1909; Gay, 1909; Ahl, 1927; Das, 1932; Sood, 1939, etc.) have described cases of abnormalities produced by caudal regeneration. Hooker (1912) studied the disposition of the nerves in the regenerated tail of *Lacerta agilis* and

\* Part of a thesis approved for the Ph.D. degree in Agra University.

Mahendra (1936) pointed out the role of the Reisner's fibre in the autotomous tail.

Although the autonomous type has been investigated in great detail, very few contributions have been made on the structure and function of the other types. Hyrtl (1853) described the vertebræ of saurian families; Mivart (1870) made scanty observations on the caudal muscles of *Chameleon parsonii*, Fischer (1907) dealt with the anomalous scalation of a regrown tail of *Agama tuberculata*, and Woodland (1920) dealt with the fossorial type *Pygopus*.

## II. MATERIAL AND TECHNIQUE

The present investigation is based on seven specimens of *Chameleon zeylanicus Laurenti*, obtained by the author from South India. One of the specimens was presented by Professor B. K. Das of Osmania University, Hyderabad, while the others were caught in Perungudi, a place ten miles from Madras.

The following methods were employed in the study:—

A. *The Skeleton*.—The skeleton of the tail was studied by three methods:

(1) *Preparations made by treating with caustic potash*.—The specimens were skinned, fixed in 90 per cent. alcohol for two or three days, and then macerated with 5 to 10 per cent. potassium hydroxide solution.

(2) *Alizarin preparations*.—This method was found especially useful in the study of very small tails, since in them the deeply stained bones can be seen clearly through the overlying semi-transparent tissues.

(3) *Serial Transverse, Sagittal and Frontal Sections*.—The material was decalcified with a mixture of 10 c.c. of strong nitric acid and 90 c.c. of 70% alcohol, sections were cut 8 or 10 microns thick and stained with Mallory's triple stain. The relation between the caudal muscles and vertebræ was studied mainly in transverse sections.

B. *Musculature*.—The caudal muscles were studied by the following methods:

(1) *Removal of the skin carefully from the underlying muscles*.—This was necessary for examining the superficial appearance of the caudal musculature and tendons.

(2) *Dissections*.—The muscles were dissected mostly under a binocular microscope, and their disposition, as well as relation to the axial skeleton, was carefully noted.

(3) *Serial Sections*.—Transverse, sagittal and frontal sections were examined, passing through both the inter and intravertebral planes.

### III. GENERAL

The tail of *Chameleon*, although it generally lies coiled up like a watch-spring unlike that of other lizards, can straighten out when necessary to secure a hold on a convenient twig or branch. In adaptation to this prehensile function, it shows numerous anatomical features of special interest, although these have so far failed to attract the attention they deserve. The only investigator who paid some attention to the anatomy of the tail of *Chameleon* was Mivart (1870), who gave a general account of the body and tail musculature in *Chameleon parsonii*.

The seven specimens, studied by the author, ranged from 5 to 7 inches in length from snout to vent while the tail in each case was a little longer. These measurements are in agreement with Smith's statement about this species, "Tail at least as long as head and body." The collection included individuals of both sexes.

The tail has a thick basal region which is strongly compressed from side to side and passes on into the long and tapering coiled portion. The latter is also compressed from side to side, but not to the same extent as the basal one. A little anterior to the end of the tail, the coils are extremely small and close and the compression almost entirely disappears.

### IV. EPITHELIUM AND LEPIDOSIS

The granules are small and tubercular. Those on the dorsal and lateral surfaces are similar to, and of the same size as, the ones on the trunk and limbs. The ventral ones are slightly smaller than these and are arranged in regularly transverse rows, the latter being continued in an annular manner on to the dorsal side. Intercalated between these annular rows, there are additional ones on the dorsal side so that the dorsal transverse rows exceed the ventral ones in number. This is evidently an adaptation to the coiled nature of the tail, since on account of such a disposition the dorsal surface is longer than the ventral one.

An examination of transverse sections of the skin (Fig. 1) shows that the granules are entirely non-imbricate. They appear as conical elevations on the epithelium with a fairly thick and laminated epidermis. The dermis is composed of a loose connective-tissue, differentiated into an outer sub-epidermal region in which the fibres are disposed vertically and a deeper layer in which the fibres run concentrically outside the musculature.



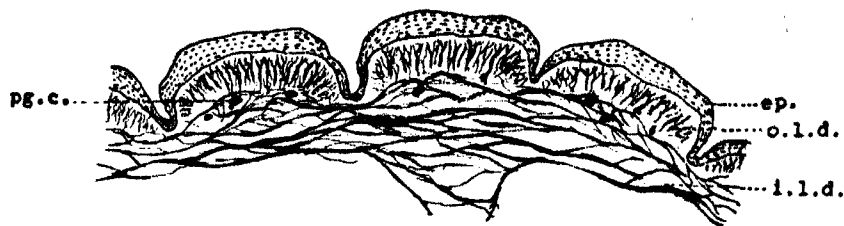


FIG. 1. Transverse Sections of the skin of *Chameleon zeylanicus* ( $\times 16$ ).  
ep., epidermis; i.l.d., and o.l.d., inner and outer layer of dermis; p.g.c., pigment cells.

A very interesting feature, discovered in sagittal sections (Fig. 2), is the fact that in correlation with their physiological requirements the dorsal and ventral portions of the skin differ from each other in the terminal part of the tail. Since the former has to provide for the stretching produced during uncoiling, it is thinner and bears flat and depressed scales. The latter,

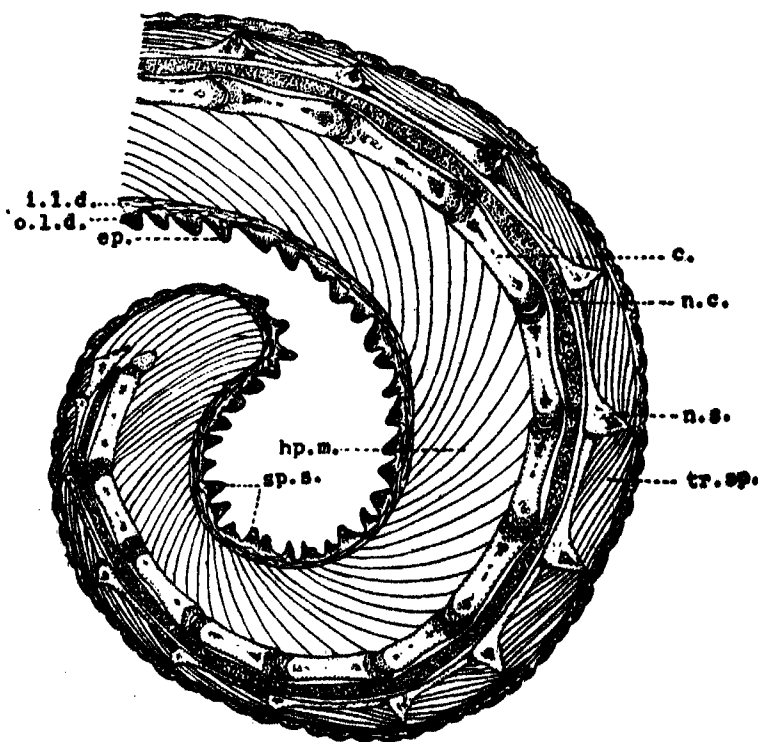


FIG. 2. Sagittal section passing through the terminal part of the tail of *Chameleon zeylanicus*. ( $\times 16$ ). c., centrum; hp.m., hypaxonic muscles; n.c., nerve cord; n.s., neural spines; sp.s., spine-like scales; tr.sp., M. transverso-spinalis; other abbreviations as in the previous figure.

on the other hand, is thick and strongly built to withstand the friction it has to bear; it possesses large spine-like scales, resembling a series of conical pegs and serving to give a firm grip on the twig.

## V. THE CAUDAL VERTEBRAE

Skiagrams and alizarin-stained preparations show that the vertebral column in the tail of *Chameleon zeylanicus* lies not exactly equidistant from the upper and lower surfaces, but rather towards the dorsal side. As we proceed towards the extremity, the vertebral column shifts more and more dorsalwards until near the end of the tail, it lies almost entirely dorsal to the caudal muscles. This is perhaps due to the peculiar watch-spring type of coiling, exhibited by the distant part of the tail.

As the caudal musculature and vertebral column are responsible for the movements performed by the tail, the vertebrae show a number of interesting adaptations. Not only is their general structure more or less modified, but the relative positions of their component parts have undergone alteration. The following details may be noted:

(1) The *zygapophyses* (Fig. 3) are extremely well developed in *Chameleon* and Mivart (1870), in describing the longissimus muscles of the trunk referred to their elongation as simulating the metapophyses of mammals. In the tail, the unusually extended post-zygapophyses, with their articular

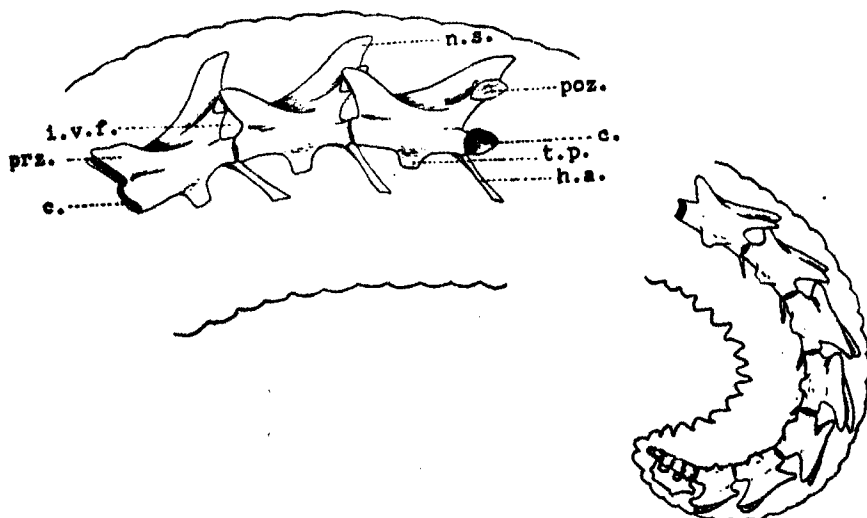


FIG. 3. Lateral view of the caudal vertebrae of *Chameleon zeylanicus* from an alizarin-stained preparation ( $\times 6$ ). *h.a.*, haemal arch; *i.v.f.*, inter-vertebral foramen; *poz.*, postzygapophysis; *prz.*, prezygapophysis; *t.p.*, transverse process; other abbreviations as in previous figures.

faeets directed downwards and outwards, fit in between (rather than overlap) the prezygapophyses of the following vertebræ, the latter facing upwards and inwards to accommodate them. Thus in a lateral view the prezygapophyses are seen externally situated to the postzygapophyses. This sort of articulation, while allowing a free up-and-down movement of the tail considerably restricts the side-to-side movements. The extraordinary elongation of the zygapophyses and the modification in the plane of their articulation are unique features, on account of which, even when the tail is fully coiled downwards, the zygapophysial articular surfaces remain in close contact with each other thereby giving a remarkable strength to the act of grasping.

(2) Correlated with the modification of the zygapophyses, the *inter-vertebral foramina* (Fig. 3) have increased in size. As shown by the skiagram and alizarin preparations, they are extraordinarily large, due probably to the above-mentioned extension of the zygapophysial processes.

(3) The *transverse processes* which are dorso-ventrally compressed project from the ventral surface of the centrum downwards rather than outwards. They are situated midway between the anterior and posterior ends of the vertebra and divide the tail unequally, as in most vertebrates, into an upper and a lower half. The upper half is occupied by the vertebral column and the epiaxonic muscles, while the lower one contains the hypaxonic musculature, the hæmal arches and the caudal vessels.

(4) The *neural spine* which is a very prominent structure in the caudal vertebræ of *Chameleon* is confined almost completely to the posterior part of the vertebral roof. It is in the form of a laterally compressed, backwardly projecting ridge, which at its anterior end slopes forwards to end approximately near the middle of the vertebra. The fact that the neural ridge is not present on the anterior half of the neural arch is perhaps a consequence partly of the close overlapping of the vertebræ and partly of the disposition of the muscle attachments.

(5) The disposition of the *centrum* in these vertebræ (Fig. 2) is also peculiar. Its anterior cup-shaped surface has the lower lip shorter than the upper and lateral ones. The posterior convexity is, accordingly, not evenly rounded but possesses a more or less distinct downward-and-backward slope in its dorsal half as compared to its ventral one. This is also an adaptation for the dorso-ventral flexion of the tail and becomes more conspicuous as we proceed backwards.

## VI. THE CAUDAL MUSCULATURE AND TENDONS

As is generally known, the somatic musculature in vertebrates is divided into two sets by a horizontal myoseptum: the epiaxonic and hypaxonic

sets. The epiaxonic set consists precaudally of three systems of longitudinal muscles divided from each other by two lines: (a) the line connecting the zygapophysial articulations with each other and (b) that connecting the successive costo-transversal articulations. These systems are called the transverso-spinalis, the longissimus and the ilio-costalis. The first occupies the spaces on either side of the neural spine between the right and left articulations of the zygapophyses. The second lies ventro-lateral to the zygapophysial articulations but dorsal to the transverse processes. The third is situated still more laterally on the dorsal part of the ribs.

The hypaxonic musculature consists of the muscle trunks connected with the ribs. In the *Urodela* there are four pairs and in *Sphenodon* six.

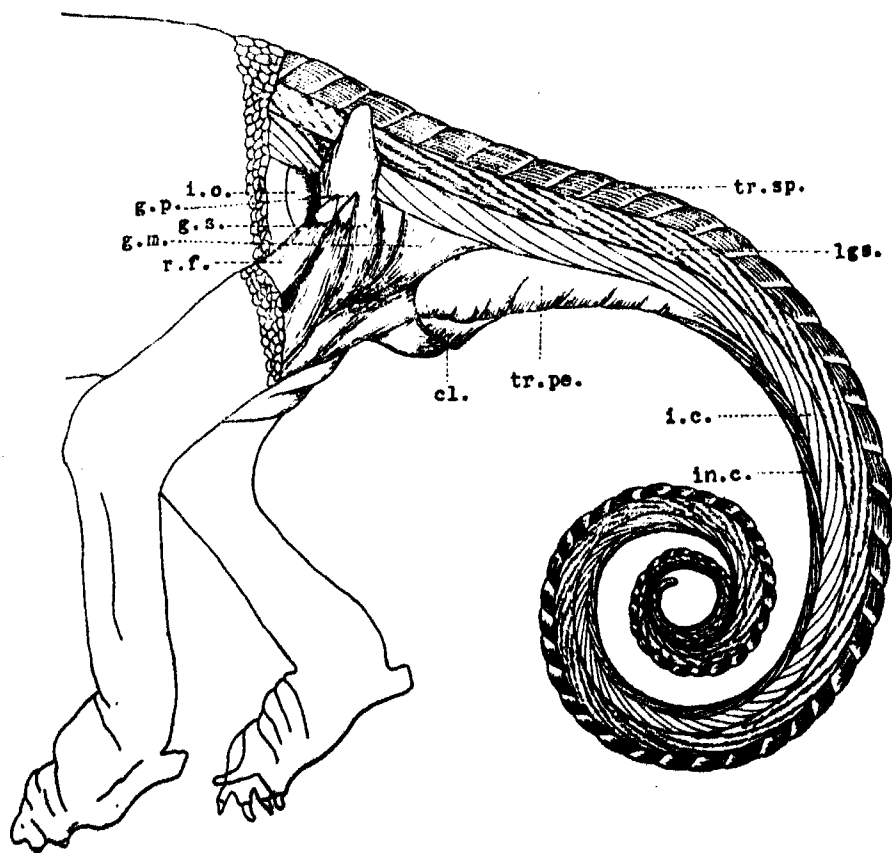


FIG. 4. The Caudal Musculature of *Chameleon zeylanicus* in surface view seen from the left side after removal of the skin ( $\times 2$ ). Cl., cloaca; g.m., M. gluteus maximus; g.p., M. gluteus primus; g.s., M. gluteus secundus; l.c., M. ilio-caudalis; in.c., M. infero-caudalis; i.o., internal oblique muscle; lgs., M. longissimus; r.f., M. rectus femoris; tr.pe., M. transversus perinei; tr.sp., M. transverso-spinalis.

Their fibres run obliquely from rib to rib although they vary in direction in different trunks.

This general plan exhibited by the trunk musculature is simplified in the caudal region on account of the absence of ribs. Not only, therefore, is the ilio-costalis system altogether absent but the number of bundles is also greatly reduced in the hypaxonic set.

In the tail of *Chameleon zeylanicus* there are four pairs of longitudinal muscles, of which two are epiaxonic and two hypaxonic. In addition to these, four more muscles occur at the base of the tail, which have already been properly described by Mivart (1870) in *Chameleon parsoni* and need not be repeated here.

When the skin is removed, the caudal musculature of *Chameleon zeylanicus* (Fig. 4) is found to be composed of longitudinally running muscle-bands which are overlaid by a large number of tendons, varying in form and strength. These originate from the longitudinal muscle trunks at regular intervals. After running for a short distance, usually for the length of three vertebræ, they get inserted on some part of a vertebra; viz., on the neural spine, zygapophysis, transverse process, or hæmal arch. This elaborate system of tendons, arising from the caudal muscle-trunks and inserted on the vertebral column, is responsible for the characteristic coiling and uncoiling of the tail. It will be described in detail later on.

The nomenclature of muscles used in the following description is the one, adopted in Ihle, Kampen, Nierstrasz and Versluy's text-book *Vergleichende Anatomie der Wirbeltiere* (Berlin, 1927) and differs from that of Mivart's work.

(1) *M. Transverso-spinalis* (upper part of *Supra-Caudal muscle* of Mivart).—This pair of muscles runs throughout the length of the tail and occupies the space between the neural ridge and the zygapophysis on each side. Superficial to these muscles, there are numerous peculiar tendons which run obliquely. A close study of them (Figs. 5 and 6) reveals that each tendon originates from the muscle close to the zygapophysis of a vertebra, runs in an upward and backward direction until it reaches the neural spine of the succeeding vertebra, and then running over the vertebral column gets inserted on the neural spine of the vertebra immediately behind this one. Thus each of these tendons when minutely observed, is found to extend over the length of two vertebræ although in a superficial view a continuous tendinous sheath appears to cover the dorsal musculature of the tail.

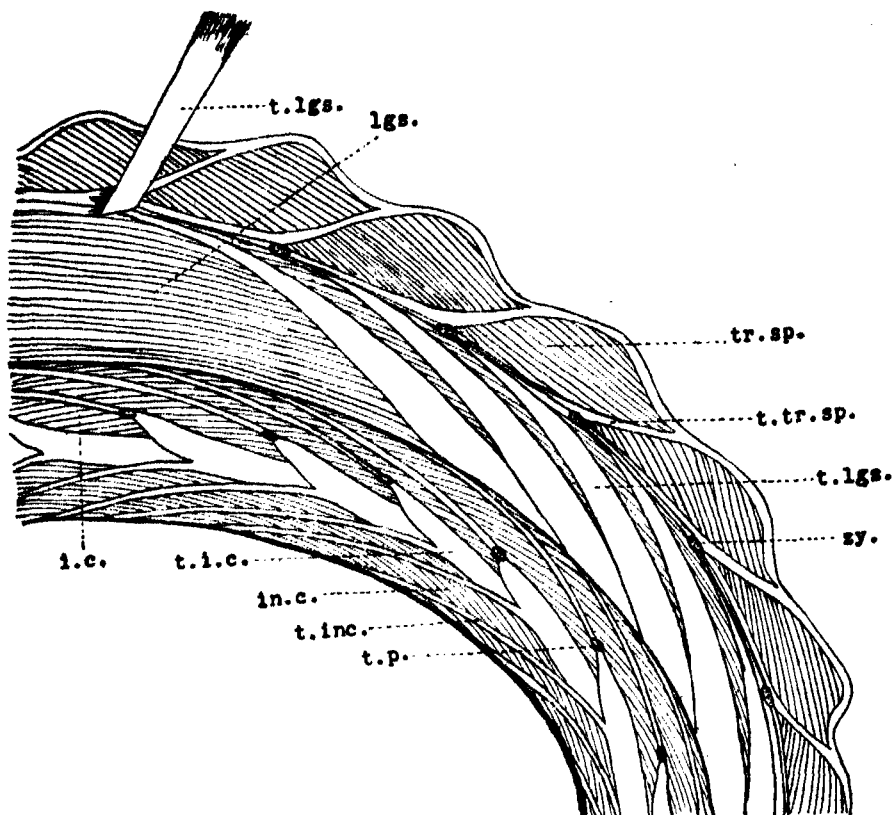


FIG. 5. A portion of the caudal musculature of *Chameleon zeylanicus*, magnified to show the disposition of muscles and tendons ( $\times 8$ ). *t.i.c.*, tendons of *M. Ilio-caudalis*; *t. inc.*, tendons of *M. Infero-caudalis*; *t. lgs.*, tendons of *M. longissimus*; *t. lgs.*, a single tendon of *M. Longissimus* dissected out from the muscle; *t. tr. sp.*, tendons of *M. transverso-spinalis*; *zy.*, point of articulation of the zygapophyses; other abbreviations as in previous figures.

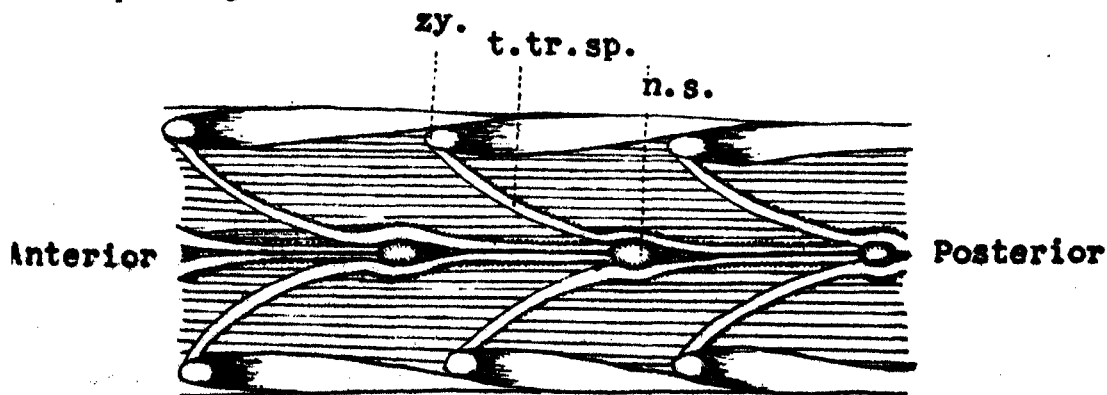


FIG. 6. Dorsal view of the caudal musculature of *Chameleon zeylanicus* after removal of the skin ( $\times 8$ ). Abbreviations as in previous figures.

(2) *M. longissimus* (= lower part of the *Supracaudal* muscle of Mivart).—Each *longissimus* muscle, which is the thickest and perhaps the strongest of all the caudal muscles, occupies the groove between the zygapophysis and the transverse process of its side and extends up to the end of the tail. It is characterized by a series of broad ribbon-like tendons which cover it throughout its extent. Each of these tendons after taking its origin from the zygapophysis of a vertebra, runs backwards and obliquely downwards for the length of about three vertebræ and finally merges into the body of the *longissimus* muscle. As the tendons are broad and overlapping, they superficially cover the whole of the muscle trunk.

The above-mentioned tendinous arrangement of *M. longissimus*, not hitherto described, has a special significance. When the muscle contracts, all the individual tendons connected with it on the one hand and with the series of caudal vertebræ on the other, are pulled simultaneously and thus the movement responsible for uncoiling is distributed throughout the whole extent of the tail. In other words, the *longissimus* muscle not only initiates but also co-ordinates the activities of all its component tendons.

An analogous case of similar coordination by a single muscle of the contractile activities performed in a number of successive segments was observed by Mosauer (1932) in Colubrid snakes. There, "some of the muscles are linked together so that they extend over more than 30 vertebræ without attachment. The purpose of this is to permit an equal bending over a considerable length of the vertebral column. If the muscles were to stretch over a few segments only, the synchronous and equal flexure would not be as well insured as by very long muscles overlapping each other by one segment only."

The transverso-spinalis and *longissimus* muscles together form the epiaxonic set. When contracted, they make each vertebra bend upwards on the preceding one and thereby lead to the uncoiling of the tail. Mivart (1870) regarded them as parts of a single muscle, the *supra-caudal*; but it is clear from their homologies, as well as from their disposition and appearance in dissections and serial sections that they are separate trunks.

(3) *M. Ilio-caudalis* (= *Ilio-caudal* muscle of Mivart).—This muscle arises partly from the ilio-sacral attachment and partly from the posterior surfaces of the transverse processes of the sacral vertebræ. According to Mivart, it may perhaps be regarded as representing a more or less backward continuation of the *Musculus Sacro-lumbalis*. It occupies the spaces lying above, below and between the transverse processes.

Each ilio-caudal muscle is divided into two parts: an upper part (*Pars dorsalis*) situated above the transverse processes, and a lower part (*Pars ventralis*) below them. Not only can we distinguish these parts by the fact that they are separated by the transverse processes, but their tendons also differ in shape and thickness, and run in opposite directions. The tendons originating from the *Pars dorsalis* are narrow and extend backwards and downwards for a length of about three vertebræ before getting inserted on the ends of the transverse processes. Those of the *Pars ventralis*, on the other hand, are much broader and stronger and run forwards and upwards for a length of about four vertebræ before their insertion.

(4) *M. Infero-caudalis* (= *Infero-caudal* muscle of Mivart).—This muscle runs all along the ventral side of the tail and is separated in the middle line from its fellow of the other side by a double-walled vertical septum which encloses the hæmal arches and vessels. Each tendon of the muscle is a thin filamentous structure which extends backwards and upwards for a length of about three vertebræ and then joins a tendon of the *Pars ventralis* of the *Ilio-caudal* muscle a little before the insertion of the latter on a transverse process.

## VII. FUNCTIONAL ADAPTATION

As already mentioned, the function of such a tail is to provide a firm grip on some convenient support by coiling around it. Consequently, the caudal vertebræ are modified in their form and articulation to perform movements on each other in a dorso-ventral plane. The latitude for lateral movements between them is considerably lessened by the peculiar disposition of the zygapophyses and each vertebra moves on its predecessor downwards rather than upwards, thereby contributing to the characteristic downward coiling of the tail. The dorsal skin, in order to provide for the necessary stretching, has wider spaces between the scales and the ventral surface bears a series of peg-shaped scales in the distal part. The musculature and tendinous arrangements are unique in the whole of Sauria. The hypaxonic set is responsible for keeping the tail in a flexed condition; and it is, on the whole, better developed in the posterior half of the tail than the epiaxonic one, since in this part the tail can be uncoiled only partially.

The epiaxonic musculature possesses a twofold disposition of tendinous insertions; that is to say, certain tendons originate from a zygapophysis, extend backwards and downwards over three vertebræ and merge into the longissimus muscle. Others, originating from a zygapophysis, run upwards and backwards to connect two successive neural spines. The contraction of these tendons draws up the vertebræ upwards on each other and thus



serves to uncoil the tail. The activity of the individual tendons is correlated by their merging into the body of a single longitudinal muscle trunk, and thus the uncoiling movement is distributed throughout the whole extent of the tail.

### VIII. SUMMARY

The *prehensile* type of tail, found in the family *Chameleonida*, is adapted for grasping supports by coiling and uncoiling in a dorso-ventral direction. It has the following features.

(a) The scales are arranged in an annular manner, the transverse rows of the dorsal side exceeding those of the ventral.

(b) The ventral epithelium differs from the dorsal in being thicker and stronger and in being provided with peg-shaped spines.

(c) The vertebral column is displaced gradually upwards as it proceeds posteriorly. The pre- and post-zygapophyses are extremely well developed, the latter fitting not over, but in between the former so that the lateral movements of the tail are restricted. The inter-vertebral foramina are large. The transverse processes which arise from the ventral part of the centrum are short and broad, lie at the middle of the vertebra and face outwards and downwards. The neural spine is a prominent structure confined almost completely to the posterior part of the vertebral roof. The anterior face of the centrum has its lower border much shorter than the upper and lateral ones.

(d) The caudal musculature, which has been described in detail, consists of longitudinally extended muscles, overlaid by numerous tendons of varying form and length.

### IX. ACKNOWLEDGMENTS

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# A SURVEY OF THE FISHERIES OF THE TUNGABHADRA RIVER\*

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## 1. INTRODUCTION

THE present paper deals with the fluvial fishes of the section of the Tungabhadra River which borders the Madras Presidency, and is based on a survey conducted by the authors from October 1943 to December 1947.

The Tungabhadra River (Fig. 1) enters the Madras Presidency from the Mysore Plateau. After forming the boundary of the Bellary District along the whole length of its western and northern sides, it empties into the Kistna a few miles south of Kurnool town. The total length of the river in the Presidency is about 300 miles. The districts of Bellary and Kurnool drain by the Tungabhadra and its three main tributaries, the *Chinna Hagari*, the *Hagari* and the *Hindri*.

The river runs through a barren tract, 940 to 1,730 feet above mean sea-level, bounded by high banks in the Madras Presidency. Navigation is difficult, as the bed is for the most part rocky. Coracles of hide or steel are the sole means of communication. Floods occur from the end of June to the end of November, when the flow of water is terrific, with a maximum discharge of about 20,800 cubic feet per second. The water is turbid and highly silt-laden. No fishery worth the name is harvested during the flood

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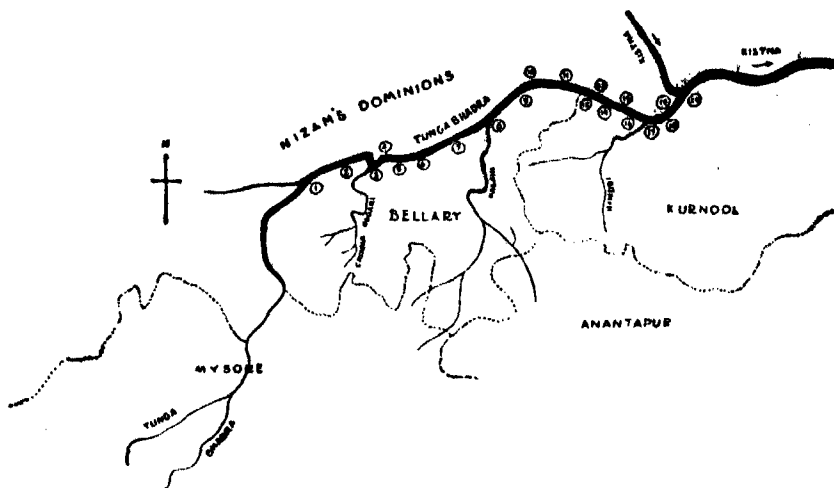


FIG. 1. The River Tungabhadra showing the Twenty Fish-breeding and Nursery Areas, located by the authors.

Scale 1 inch : 30 miles.

- |                   |                  |                |
|-------------------|------------------|----------------|
| 1. Hampasagaram.  | 8. Halekote.     | 15. Nidusuru.  |
| 2. Bommasamudram. | 9. Tumbiganuru.  | 16. Kallur.    |
| 3. Vallabhapuram. | 10. Rampuram.    | 17. Jolapuram. |
| 4. Mallapuram.    | 11. Singavaram.  | 18. Podur.     |
| 5. Hospet.        | 12. Kottamotta.  | 19. Alturu.    |
| 6. Hampi.         | 13. Sunkesula.   | 20. Sangam.    |
| 7. Desanuru.      | 14. Devanmadugu. |                |

season, but early in December when the floods subside and the water becomes clear, fishing commences. During the summer months, from March to June, the river practically dries up, leaving certain deep pools, 15 to 30 feet in depth, connected by a sluggish stream. These afford shelter to fishes, and consequently the fisherfolk reap a good summer harvest of large-sized fishes from them. There are fourteen small anicuts across the river, the most important being the one at Sunkesula, which diverts water into the Kurnool-Cuddappah canal for irrigation purposes.

## 2. FISHING METHODS

There are twenty-one fishing villages along the river bank, with an average population of 200 in each. The fishing methods are primitive. The common craft used for ferrying is the leather covered basket boat (*coracle*). The important types of fishing tackles used in the river are cast nets, drag nets, stake nets, long lines, rod and line, and nooses. The mesh-size of the nets ranges from  $1/8$  to 3 inches. The small-meshed nets and the nooses are fixed across rapids among boulders to capture minnows and bottom feeders, such as, *Discognathus lamta* and *Glyptothorax lonah*, which are capable of

Family	Scientific Name of Fish			Place of Collection and Name of District in Province or State	Recorded by (initials only)	Local Name
	Genus	Species	Author			
<i>Neopteridae</i> <i>Madacambelidae</i>	<i>Nadopterus</i>	<i>N. nadopterus</i>	Pallas	Sunkesula, Kurnool, Madras	P.I.C. & G.K.K.	Olligathatha
	<i>Macrognathus</i>	<i>M. aculeatus</i>	Bloch	Sunkesula, Kurnool, Madras	P.I.C. & G.K.K.	Chinnakontimukku
	<i>Madacambelus</i>	<i>M. armatus</i>	Lacepede	Shimoga, Mysore	S.L.H.	Kontimukku
<i>Anguillidae</i>				Hebbe, Kadur, Mysore	B.S.B. & A.S.R.	do
				Thungabhadra, Mysore	B.S.B.	
				Jolapuram, Kurnool, Madras	P.I.C. & G.K.K.	
<i>Cyprinidae</i>				Alampur, Hyderabad	M.R.	do
	<i>Anguilla</i>	<i>A. bengalensis</i>	Hamilton	Sunkesula, Kurnool, Madras	P.I.C. & G.K.K.	Malagu
	<i>Labeo</i>	<i>L. fimbriatus</i>	Hamilton	Tungabhadra, Mysore	B.S.B.	Erragenda
				Jolapuram, Kurnool, Madras	P.I.C. & G.K.K.	
				Tungabhadra, Mysore	B.S.B.	
				Alampur, Hyderabad	M.R.	
				Sunkesula, Kurnool, Madras	P.I.C. & G.K.K.	..
				Hariharpur, Kadur, Mysore	B.S.B. & A.S.R.	..
				Tungabhadra, Mysore	B.S.B.	Kakigenda
				Tungabhadra, Mysore	B.S.B.	..
				Kottamotta, Kurnool, Madras	P.I.C. & G.K.K.	..
				Tungabhadra, Mysore	B.S.B.	..
				Shimoga, Mysore	S.L.H.	..
				Hariharpur, Kadur, Mysore	B.S.B. & A.S.R.	..
				Tungabhadra, Mysore	B.S.B.	..
				Shimoga, Mysore	S.L.H.	Pariga
				Hariharpur, Kadur, Mysore	B.S.B. & A.S.R.	
				Tungabhadra, Mysore	B.S.B.	
				Sunkesula, Kurnool, Madras	P.I.C. & G.K.K.	Peddapoliga
				Hebbe, Kadur, Mysore	B.S.B. & A.S.R.	
				Alampur, Hyderabad	M.R.	
				Rampuram, Kurnool, Madras	P.I.C. & G.K.K.	Chettrupariga
				Shimoga, Mysore	S.L.H.	
				Hariharpur, Kadur, Mysore	B.S.B. & A.S.R.	
				Alampur, Hyderabad	M.R.	
				Singavaram, Kurnool, Madras	P.I.C. & G.K.K.	Pariga
				Shimoga, Mysore	S.L.H.	
				Hariharpur, Kadur, Mysore	B.S.B. & A.S.R.	
				Devamadugu, Kurnool, Madras	P.I.C. & G.K.K.	





Family	Scientific Name of Fish			Place of Collection and Name of District in Province or State	Recorded by (initials only)	Local Name
	Genus	Species	Author			
Cobitidae	<i>Rohita</i>	<i>R. cotio</i>	Hamilton	Alampur, Hyderabad	M.R. & G.K.K.	Guniguppidiigada
	<i>Eomus</i>	<i>R. ogilbii</i>	Sykes	Sangam, Kumool, Madras	P.I.C. & G.K.K.	..
		<i>E. danicus</i>	Hamilton	Hebbe, Katur, Mysore	B.S.B. & A.S.R.	Attapariga
		<i>E. barbotus</i>	Jerdon	Sunkesula, Kumool, Madras	P.I.C. & G.K.K.	..
	<i>Discognathus</i>	<i>D. lamia</i>	Hamilton	Shimoga, Mysore	S.L.H.	Rathigoraka
		<i>G. bicornuta</i>	Rao	Sunkesula, Kumool, Madras	P.I.C. & G.K.K.	..
		<i>G. jerdoni</i>	Day	Shimoga, Mysore	S.L.H.	..
	<i>Aspidoparia</i>	<i>A. morar</i>	Day	Hariharpur, Katur, Mysore	B.S.B. & A.S.R.	Rathigoraka
			Day	Shimoga, Mysore	S.L.H.	..
			Day	Hebbe and Hariharpur, Katur, Mysore	B.S.B. & A.S.R.	..
	<i>Catla</i>	<i>C. catla</i>	Hamilton	Jolapuram, Kumool, Madras	P.I.C. & G.K.K.	Akibedsa
			Cuvier and Valenciennes	Alampur, Hyderabad	M.R. & G.K.K.	..
			Hamilton	Sunkesula, Kumool, Madras	P.I.C. & G.K.K.	Botcha
	<i>Labeo</i>	<i>L. algar</i>	Hamilton	Sunkesula, Kumool, Madras	M.R.	Kotipai
			Hamilton	Shimoga, Mysore	P.I.C. & G.K.K.	..
			Hamilton	Sunkesula, Kumool, Madras	B.S.B. & A.S.R.	do
	<i>Amblypharyngodon</i>	<i>A. mola</i>	Hamilton	Alampur, Hyderabad	P.I.C. & G.K.K.	Akibedsa
			Sykes	Sunkesula, Kumool, Madras	P.I.C. & G.K.K.	Thalarigada
			Hamilton	Devanadaga, Kumool, Madras	P.I.C. & G.K.K.	Kotipai
	<i>Osteochilus</i>	<i>O. thomasi</i>	Day	Shimoga, Mysore	S.L.H.	..
			Day	Hebbe and Hariharpur, Katur, Mysore	B.S.B. & A.S.R.	Nagendran
			Day	Alampur, Hyderabad	M.R.	..
	<i>Nemachilichthys</i>	<i>N. shimogensis</i>	Rao	Sunkesula, Kumool, Madras	P.I.C. & G.K.K.	Olasa
			Rao	Shimoga, Mysore	P.I.C. & G.K.K.	..
			Rao	Hebbe, Katur, Mysore	S.L.H.	..
	<i>Nemachilus</i>	<i>N. betius</i>	Hamilton	Sunkesula, Kumool, Madras	B.S.B. & A.S.R.	Olasa
			Amundate	Shimoga, Mysore	P.I.C. & G.K.K.	..
			Day	Shimoga, Mysore	S.L.H.	..
	<i>Lepidocphthalichthys</i>	<i>N. kaimachari</i>	Hora	Jolapuram, Kumool, Madras	P.I.C. & G.K.K.	Asara
			Hora	Shimoga, Mysore	S.L.H.	..
			Cuvier and Valenciennes	Shimoga, Mysore	P.I.C. & G.K.K.	..



attaching themselves to rocks and stones by means of suckers and other adhesive organs. The methods of fishing employed here are very similar to those in the Kangra Valley described by Hora (1926). Young ones of the major food fishes are also captured with these implements. Due to the destructive methods of fishing, the fishery has considerably dwindled resulting in a poor annual harvest of about 50,000 maunds in the entire river stretch. About 60 per cent. of the catch is marketed in the Hyderabad State.

### 3. LIST OF FISHES COLLECTED

A list of 89 fishes collected from the Tungabhadra, including those recorded by Hora (1937), Bhimachar and Rau (1941), Bhimachar (1942) and Rahimullah (1943), is given above.

### 4. NOTES ON BIONOMICS

The majority of the fishes breed when the river is in floods; it is then that they show limited local migration in search of favourable breeding grounds. Spawning of *Catla catla* and *Labeo fimbriatus* were observed along the river margin near Kottamotta, Devanmadugu and Kallur in July 1945, August 1946 and September 1947. The spawning grounds are shallow marginal areas, two to four feet deep, with submerged and emergent vegetation such as *Colocasia antiquorum*, *Cyperus distans*, *C. exaltus*, *Herpestis monniera*, *Lagarosiphon roxburghii*, *Nipa fruticans*, *Panicum muticum*, *Polygonum tomentosum*, *Typha elephantina* and *Vallisneria spiralis*.

The fishery of *Labeo fimbriatus* among the carps, is the most important. This species frequents the deep pools in the course of the river. An analysis of its stomach-contents revealed the following organisms:

*Desmids and diatoms.*—*Closterium*, *Cocconeis*, *Cyclotella*, *Cymbella*, *Desmidium*, *Eunotia*, *Fragilaria*, *Gomphonema*, *Pinnularia*, *Stauronets*, *Suriella*, *Synedra* and *Tabellaria*;

*Algae.*—*Ankistrodesmus*, *Aphanocapsa*, *Spirogyra* and *Ulothrix*;

*Protozoa.*—*Eudorina*, *Pandorina*, *Volvox* and *Vorticella*;

*Rotifera.*—*Hydatina* and *Rotifer*;

*Crustacea.*—*Cypridopsis*, *Daphnia*, *Diaptomus* and *Nauplius*; and

*Insecta.*—*Nepa* and *Notonecta*.

The fry of this species could be distinguished by the ventrally situated mouth which bears folded lips and strainers, a black shoulder-spot and a grey circular patch on the caudal peduncle. The food of the fry is similar to that of the adult, but lacks crustacean and insect remains. It was found that the shoulder spot and the caudal spot disappear when the fry attain a length of two and three inches respectively.

The larger-sized carps whose fry and fingerlings are common in the river from August to December are *Labeo fimbriatus*, *L. calbasu*, *Barbus tor*, *Cirrhitina reba*, *C. fulungee*, *Thynnichthys sandkhol* and *Osteochilus thomassi*. The occurrence of the last species in this area has been regarded as an evidence of Malayan affinities in the fresh-water fauna of Peninsular India (Hora, 1942 and 1944; and Bhimachar, 1945). In the Tungabhadra river, *Osteochilus thomassi* attains a size of 24 inches, frequenting the deep pools in the course of the river. It is a plankton-feeder (Chacko, Venkatraman and Kuriyan, 1947); and its food consists of *Closterium*, *Cosmarium*, *Fragilaria*, *Gomphonema*, *Melosira*, *Navicula*, *Nitzschia*, *Pinnularia*, *Stauroneis*, *Synedra* and *Suriella*. Copepods and their eggs as well as fine sand particles were also observed in the stomach of a few specimens. The fish attains maturity when it is 10 to 12 inches in length. The mature egg is 1.14 to 1.17 mm. in diameter. The breeding season extends from June to September, when the breeders move up the river in small shoals of about fifty in search of shady gravelly banks with a strong current of water. Experiments conducted have proved that this fish is well suited for pond culture and that it attains a size of 9 to 12 inches in the first year of its life.

The large cat-fishes, *Mystus aor*, *M. seenghala* and *Bagarius bagarius* thrive well in this river. Their maximum sizes as observed by us are 40, 48 and 85 inches respectively. The specimen of *Bagarius bagarius* was 310 pounds in weight and had to be carried by four men to the nearest market, where it was filleted and sold for Rs. 75. *Mystus aor* and *M. seenghala* have been observed breeding in the months of September to December in the river above Sunkesula Anicut. Our observations on their breeding habits confirm those of Raj (1940) in the Cauvery river. These fish make circular depressions amidst small rocks with their pectoral, ventral and anal fins and the lower caudal lobe; and the male guards the brood.

The breeding of the large murrel, *Ophicephalus marulius*, in the Tungabhadra near Hampasagaram, Vallabhapuram, Hampi, Rampuram and Podur from October to December, confirm the observations on this species (Chacko and Kuriyan, 1947) in the Cauvery river. Cup-like clearings with broods were discovered along the sheltered woody margin of the river, as in the case of *Ophicephalus striatus* (Wildey, 1910; Raj, 1916; Khan, 1924 and 1926; Bhattacharya, 1946) and *Ophicephalus punctatus* (Raj, 1916; Khan, 1924; Narayan Rao and Seshachar, 1927).

#### 5. SEED COLLECTION

We have located twenty breeding and nursery areas since 1943 which are now being systematically exploited for stocking departmental farms

and provincial waters in the Ceded Districts of Madras Province (Fig. 1). These nursery areas are shallow and shady sections of the river margin, where the water is slightly brownish with a temperature of 22–28° C.

The following are the details of 65,000 young *Catla catla*, 140,000 of *Labeo fimbriatus*, 5,000 of *L. calbasu*, 27,000 of *Cirrhina fulungee*, 22,000 of *C. reba*, 11,000 of *Osteochilus thomassi*, 6,000 of *Barbus tor*, 5,000 of

Name of nursery area	Species	No. collected	Size in cm.
Rampuram	<i>Barbus tor</i>	1,000	6–12
	<i>Ophicephalus marulius</i>	3,000	4–8
Singavaram	<i>Catla catla</i>	10,000	2–6
	<i>Labeo fimbriatus</i>	16,000	2–8
	<i>Cirrhina fulungee</i>	5,000	3–7
	<i>C. reba</i>	2,000	3–6
	<i>Barbus tor</i>	2,000	6–12
	<i>Mystus seenghala</i>	500	4–8
Kottamotta	<i>Catla catla</i>	3,000	2–6
	<i>Labeo fimbriatus</i>	20,000	2–8
	<i>L. calbasu</i>	1,000	4–6
	<i>Cirrhina fulungee</i>	8,000	3–7
	<i>C. reba</i>	8,000	3–6
	<i>Barbus tor</i>	3,000	6–12
Sunkesula	<i>Mystus seenghala</i>	3,000	4–8
	<i>Catla catla</i>	20,000	2–10
	<i>Labeo fimbriatus</i>	70,000	2–10
	<i>L. calbasu</i>	1,000	4–6
	<i>Cirrhina fulungee</i>	10,000	3–7
	<i>C. reba</i>	6,000	3–8
	<i>Osteochilus thomassi</i>	4,000	6–10
Devamadugu	<i>Thynnichthys sandkhol</i>	2,800	8–10
	<i>Catla catla</i>	7,000	2–12
	<i>Labeo fimbriatus</i>	10,000	2–10
	<i>L. calbasu</i>	3,000	4–6
	<i>Cirrhina fulungee</i>	2,000	3–6
	<i>Osteochilus thomasi</i>	2,000	6–10
Nidusuru	<i>Thynnichthys sandkhol</i>	2,000	8–10
	<i>Catla catla</i>	4,000	2–8
Kallur	<i>Labeo fimbriatus</i>	6,000	2–10
	<i>Catla catla</i>	4,000	2–6
Jolapuram	<i>Labeo fimbriatus</i>	4,000	2–8
	<i>Catla catla</i>	15,000	3–8
	<i>Labeo fimbriatus</i>	8,000	2–10
	<i>Cirrhina fulungee</i>	2,000	3–8
	<i>C. reba</i>	6,000	3–8
	<i>Osteochilus thomassi</i>	4,000	6–10
Podur	<i>Thynnichthys sandkhol</i>	1,000	8–10
	<i>Catla catla</i>	2,000	2–6
	<i>Labeo fimbriatus</i>	6,000	2–10
	<i>Osteochilus marulius</i>	1,000	6–10
	<i>Ophicephalus marulius</i>	2,000	4–8

*Thynnichthys sandkhol*, 5,000 of *Ophicephalus marulius* and 3,500 of *Mystus seenghala* that have been so far collected by us from the river and stocked in other inland waters.

## 6. ACKNOWLEDGMENTS

We are grateful to Dr. T. J. Job, D.Sc. (formerly Deputy Director of Fisheries, Madras), for his kind encouragement and constant guidance, to Janab Syed Hussain, Assistant Inspector of Fisheries, for his assistance in the survey, and to Professor Beni Charan Mahendra for valuable suggestions and criticism.

## 7. SUMMARY

A survey of the fluvial fisheries of the Tungabhadra River with special reference to the breeding and nursery areas of the major food fishes has been conducted. Sixty-seven species of fishes have been found by the authors, of which nineteen are new records. Breeding and food-habits of the more important fishes have been observed.

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# TRACE ELEMENT NUTRITION OF FUNGI\*

## I. The Effect of Boron, Zinc and Manganese on *Fusarium* Species

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### INTRODUCTION

FUNGI are responsible for at least two important soil processes—rapid decomposition of complex organic substances and assimilation of soluble inorganic nitrogen compounds and minerals. Most fungi are powerful cellulose decomposers, thus Daszewska (1913) established *Verticillium cellulosa*, *V. glaucum*, *Sporotrichum olivaceum* and various other *Sporotricha*, *Fusaria*, *Monosporia*, *Alternaria* and *Monilia* as the strongest cellulose decomposing fungi in the soil. *Fusarium vasinfectum* and other species of *Fusarium* were found capable of transforming 50 to 80% of the cellulose in the form of filter-paper into soluble form (Appel and Schikhorra, 1906). Waksman (1927, 32) brought out the conception of a basic fungus flora comprising of *soil inhabitants* among which may be recognized *soil invaders*. That the wilt producing *Fusaria* can be included in the *soil inhabitant* group has been proved by a number of workers and substantial evidence on their ability to colonize and decompose normal wheat straw when buried in soil was shown by Sadasivan (1939). Several workers in this laboratory have isolated a number of species of *Fusarium* particularly, *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* from arable soils collected from cotton, pigeon-pea and paddy-grown fields respectively which have a wilt-sick history in the Coimbatore District, South India. It is likely that they perennate depending for their nutrition on both the organic substrata and the inorganic salts of the soil. The precise part played by inorganic nutrients comprising of the macro- and micro-elements in maintaining at an optimum level the saprophytic phase of this inhabitant class of fungi has not been clearly understood. The first practical evidence of the ability of soil fungi to compete with the growing crop for the available supply of micro-element was given by Millikan (1938, 42) wherein he demonstrated that soil micro-organisms may compete with wheat crop for the available supply of minor elements and also the addition of zinc brought about the control in 'foot-rot' of wheat caused by a number of fungi like *Helminthosporium sativum*,

\* Formed part of a thesis accepted for the M.Sc. degree of the University of Madras.



*Curvularia ramosa*, *Fusarium culmorum* and *Rhizoctonia Solani*. Growth response by *Aspergillus niger* in pure culture in the presence of micro-elements has been shown by Steinberg (1919, 20, 34, 35, 36 a, b, 38 and 39). Stiles (1946) listed micro-elements that had favourable effect on different species of fungi of particular interest being the effect of zinc, manganese, copper, molybdenum and gallium. Dry weights of fungal mats indicated response of the fungus to the element added. Working with *Phymatotrichum omnivorum*, Rogers (1938) established high toxicity for copper and mercury at very low concentrations but zinc and manganese stimulated growth even at high concentrations. Blank (1941) working with the same fungus observed increased mat weight with the addition of iron, manganese or zinc to the cultures. Inhibition to growth of varying degrees occurred with aluminium, boron, cobalt, cadmium, fluorine, iodine, lithium, molybdenum and nickel. The first four elements mentioned above were, however, found to be non-essential. The purport of this investigation was mainly to determine quantitatively the growth response of three species of *Fusarium*, viz., *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* to micro-elements such as boron, zinc and manganese contained in liquid culture media. These fungi were specially chosen for this investigation since it was thought that their response towards micro-elements would open up a new line of attack on the admittedly difficult field problem of the wilt mechanism in plants.

#### MATERIAL AND METHODS

To obtain the results on quantitative estimation of fungal mats with reasonable degree of accuracy it was considered desirable to grow the fungi in liquid media. Hence, the media, Barne's, Coon's, glucose, Czapek's, Richard's, Horne and Mitter's and potato-dextrose were used with the elimination of agar (Rawlins, 1933). The glassware employed were Pyrex Erlenmeyer flasks and their cleaning and preparation of the media were carried out following standard mycological methods.

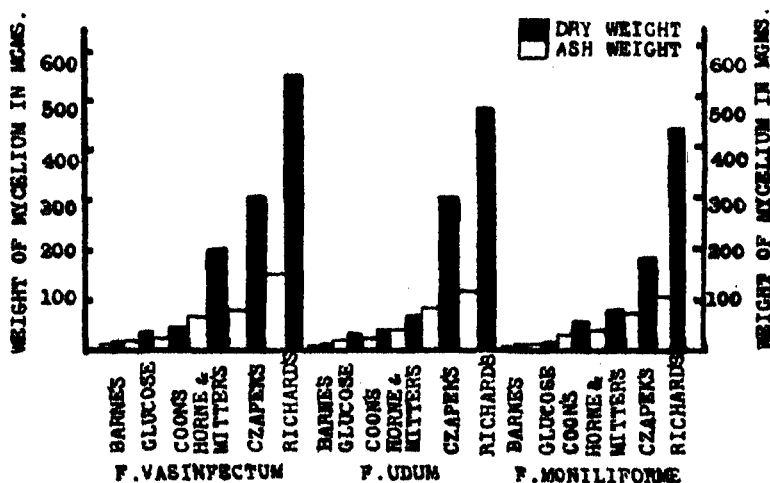
Cultures of *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* kindly supplied by the Government Mycologist, Coimbatore, were tested for their pathogenicity in this laboratory and grown in Petri dishes containing potato-dextrose agar. Young cultures from Petri dishes formed the source of inoculum; cork borer discs of agar containing the fungal mat were cut out from the periphery of the growing colony each disc having a diameter of 3 mm. 3 to 5 replicates were maintained, incubated at temperatures varying from 26 to 32° C. for the desired period. Fungal mats were filtered and collected on previously weighed filter-papers, washed with several changes of distilled water and dried to constant weights in a hot air oven at 70 to

80° C., due correction being given for the weight of the filter paper. The dry mats after preliminary ashing in a Bunsen flame were incinerated in a muffle furnace.\* The temperature was gradually raised to 500° C. and maintained for half an hour. After cooling in a desiccator the ash was weighed and the correction for the ash weight of the filter-paper made.

Double distilled water used in the micro-element experiments was distilled in a pyrex glass still. Inorganic salts employed were manufactured by Merck & Co., and they were purified and tested with dithizone for the presence of boron, zinc and manganese according to the methods suggested by Stout and Arnon (1939). Different concentrations ranging from 0.025 p.p.m. to 750 p.p.m. of boron, zinc and manganese as boric acid, zinc sulphate and manganese sulphate were added separately to aliquots of Richard's medium which was found to be the best basic medium where optimum and uniform mat production took place.

#### EXPERIMENTAL RESULTS

The selection of a synthetic liquid medium for the growth of the three species of *Fusarium* under study wherein micro-elements can be added, was first attempted and the results are diagrammatically presented in Text-Fig. 1.

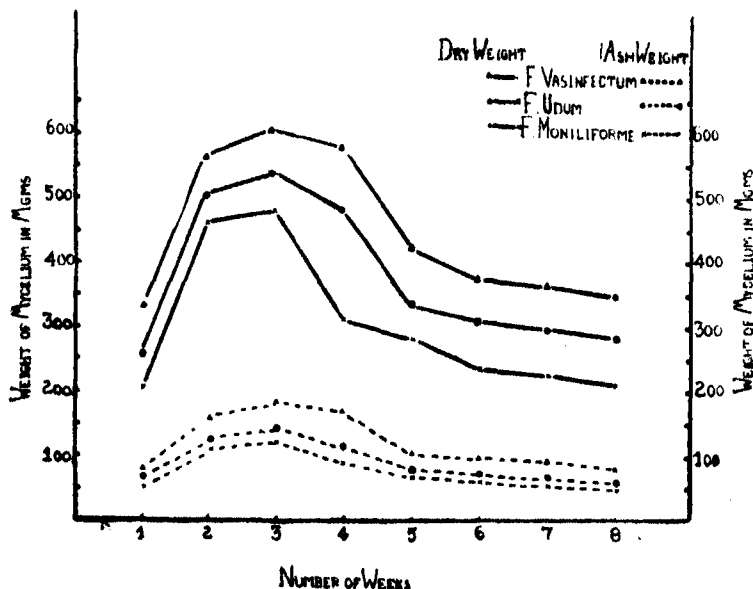


TEXT-FIG. 1. Shows mat production by three *Fusarium* spp., in different liquid media.

\* Considerable difference of opinion exists in literature on the desired temperature of incineration likely to give the most accurate results in ash weights. Experiments were performed here under pyrometer controlled incineration and it was finally decided to ash all fungal mats at 500° C. for half an hour, which time and duration indicated reasonably comparable and consistent results.

The results showed that *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* favoured media with high C/N ratio as evidenced by the highest dry and ash weights obtained in all three cases in Richard's medium. Richard's medium was therefore, used throughout this investigation on the role of minor elements in fungal nutrition.

Prior to the large-scale experimentation on the response of the fungi to minor elements, the period of incubation in liquid nutrient media necessary for the optimum production of dry and ash weights of the three fungi was ascertained. The results are summarised in Text-Fig. 2.

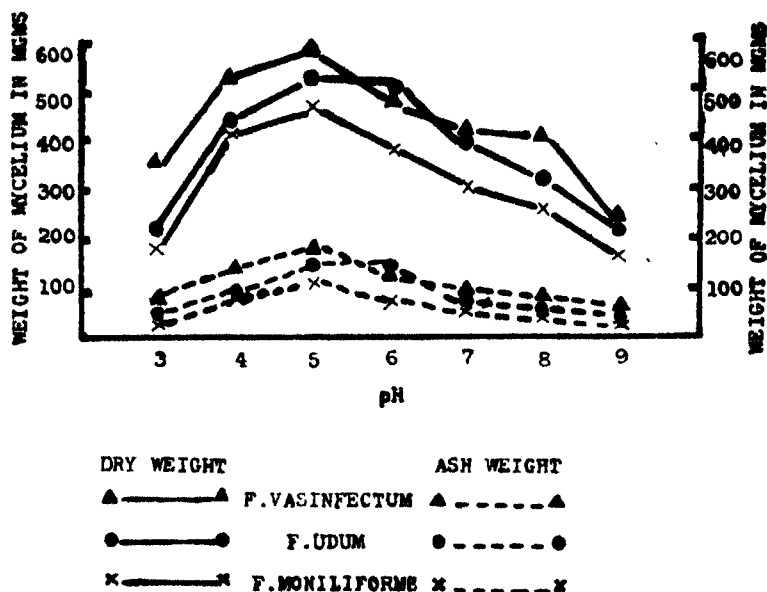


TEXT-FIG. 2. Indicates the weekly growth rate of the three Fusaria.

The mats of all the three species of *Fusarium* attained maximum dry and ash weights at the end of three weeks in Richard's medium thus pointing out the necessity of a three-week incubation in all future experiments.

The influence of hydrogen-ion concentration of the medium on the three species was undertaken with a view to determine the exact pH of the liquid media necessary for optimum development. The pH values were tested with the help of a Hellige Comparator and the results are summarised in Text-Fig. 3.

Fungi grew well over a wide range of hydrogen ion concentration and pH 5 was found to be the optimum for all the species both for dry and ash weight production.



TEXT-FIG. 3. Shows the effect of pH of the medium on the growth of *F. vasinfectum*, *F. udum* and *F. moniliforme*.

Neal (1927) working with *F. vasinfectum* observed the best growth of the fungus judged by dry weight production at pH levels 3, 4, 4.5 and 5.5 but with the strain of *F. vasinfectum* used here, pH 5 showed marked growth response over the other pH values tried (the present observations were on dry and ash weight bases of the fungus) and it is noteworthy that the response was similar for all the three fungi.

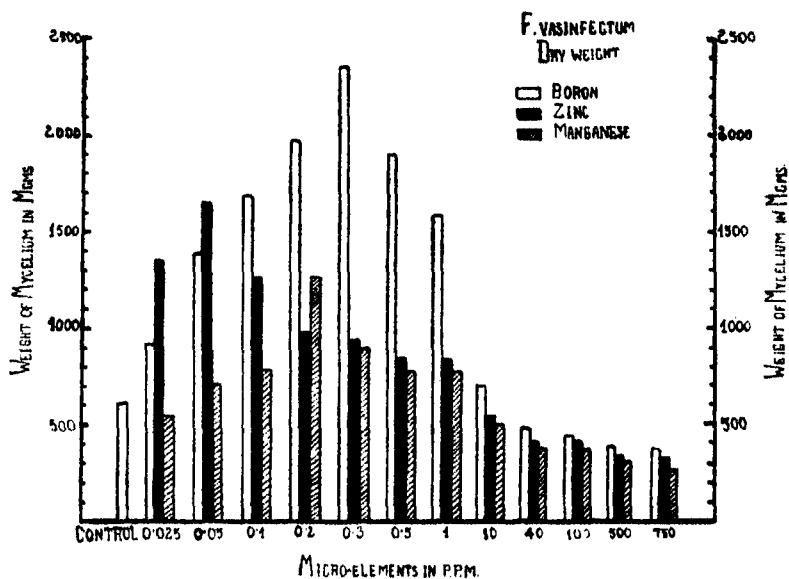
Growth response of *F. vasinfectum*, *F. udum* and *F. moniliforme* to boron, zinc and manganese, was experimented upon; dry and ash weights were recorded at different levels of micro-element and the results are presented in Text-Figs. 4 to 9.

1. Fungi grew well at all concentrations ranging from 0.025 to 750 p.p.m.

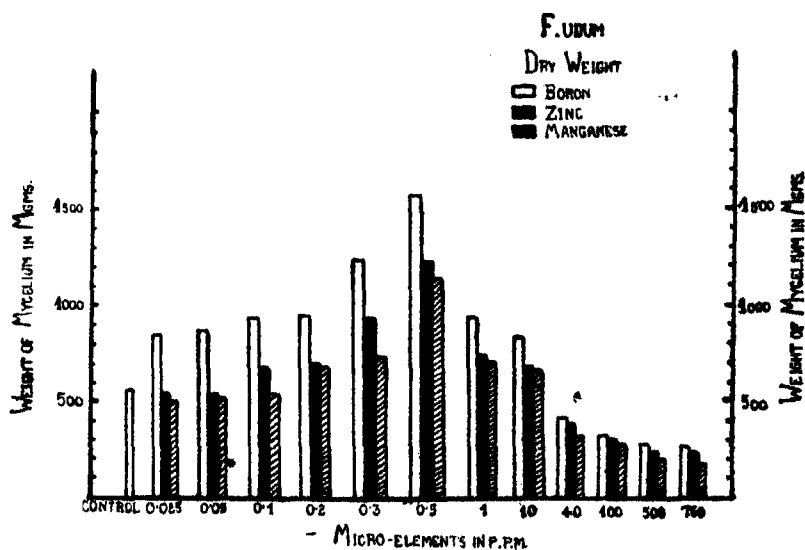
2. The lower concentrations of micro-element gave better weights (dry and ash) than the higher concentrations. Weights decreased with increase of concentration. Dry weights from 40 to 750 p.p.m. in all the fungi and the ash weights from 10 to 750 p.p.m. in *F. vasinfectum* and 40 to 750 p.p.m. in *F. udum* and *F. moniliforme* were below those of the control.

3. Boron gave highest dry weights of all the three elements. Zinc and manganese followed it respectively. Ash weights on the other hand,

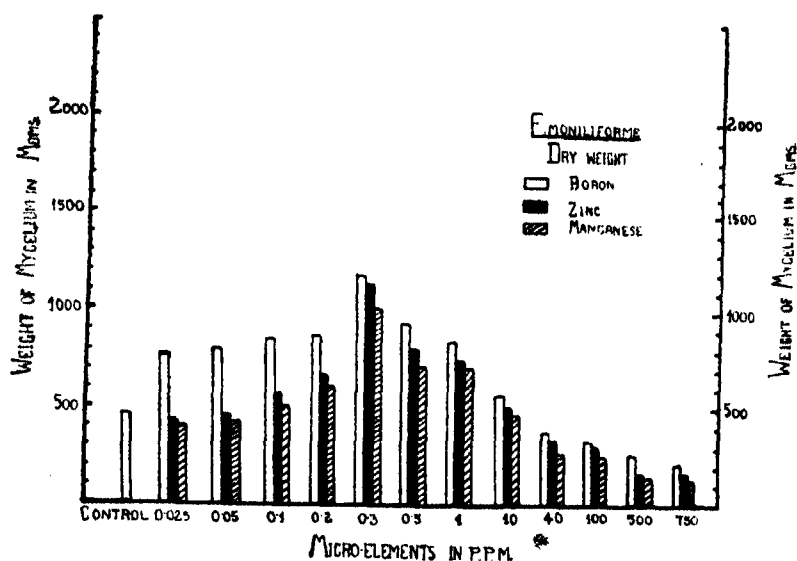
were best in plus zinc cultures and the comparative yield of ash weights were plus zinc > plus manganese > plus boron.



TEXT-FIG. 4.

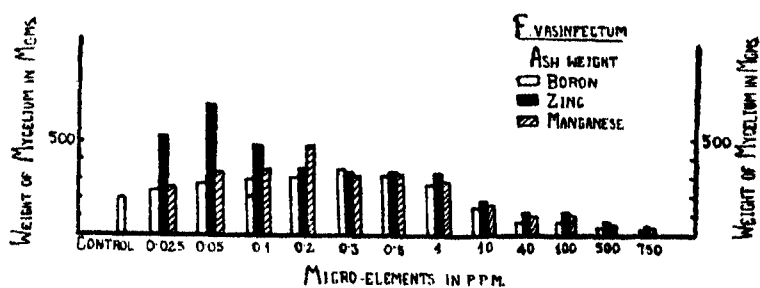


TEXT-FIG. 5.

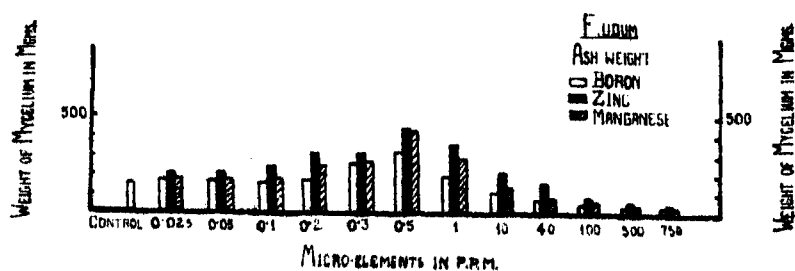


TEXT-FIG. 6.

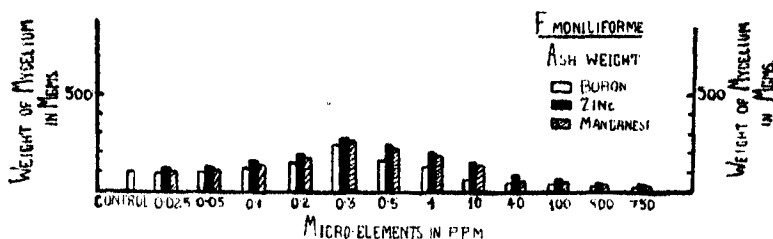
TEXT-FIGS. 4 to 6. Show the trace element response of *F. vasinfectum*, *F. udum* and *F. moniliforme* respectively to different concentrations of boron, zinc and manganese.



TEXT-FIG. 7.



TEXT-FIG. 8.



TEXT-FIG. 9.

TEXT-FIGS. 7 to 9. Show the ash weights of *F. vasinfectum*, *F. udum* and *F. moniliforme* respectively to different concentrations of boron, zinc and manganese.

4. Micro-element necessary for optimum dry and ash weight production varied with the fungus as given below:—

*F. vasinfectum*.—Optimum dry and ash weight production with boron, zinc and manganese was at 0.3, 0.05 and 0.2 p.p.m. respectively and it is interesting to note that this tendency ran parallel in the ash weight production.

*F. udum*.—Optimum dry and ash weight production was at 0.5 p.p.m. for all the three elements.

*F. moniliforme*—Addition of 0.3 p.p.m. of each of the three elements to the basic medium produced optimum dry and ash weights.

Thus variation in the response to the doses of micro-elements contained in the medium as indicated by dry and ash weights were best seen in *F. vasinfectum*. This difference in dry and ash weights exhibited by the three fungi may possibly be due to their ability to absorb more inorganic material in the presence of zinc which is retained in the ash resulting in the heavy ash weights.

In the presence of boron probably organic material is taken up which volatilizes on ashing resulting in low ash weights.

That this difference in the ash weights between the boron and zinc added cultures was probably due to the accumulation of larger weights of inorganic salts in zinc added cultures has been further clarified by recent experimentation and two main inorganic salts present in Richard's medium (in which the micro-elements, boron and zinc, were added), viz., potassium and magnesium were estimated. The methods used were those of Chapman (1947) and Scott (1939). The results are summarised in Tables I and II below:

The results show that there is a distinctly increased potassium and magnesium accumulation in the ash of zinc added cultures. Secondly, at

TABLE I

*Weight of potassium in mgms. as  $K_2O$  and magnesium as Mg from 20 mgm. samples of ash taken from boron and zinc added cultures*

Levels of micro-element added	POTASSIUM (mgms.)		MAGNESIUM (mgms.)			
	Micro-element added		Control	Micro-element added		Control
	Boron	Zinc		Boron	Zinc	
0.025 p.p.m. ..	2.7488	3.4075		1.638	1.648	
Optimum (0.3 p.p.m. B and 0.05 p.p.m. Zn)	2.4713	2.7975	2.6141	3.605	4.369	2.642
750 p.p.m.	2.2328	2.4829		1.855	2.049	

TABLE II

*Potassium and magnesium figures given in Table I are added to each other and presented as follows*

Levels of micro-element added	Micro-element added		Control
	Boron	Zinc	
0.025 p.p.m. ..	4.3868	5.0555	
Optimum (0.3 p.p.m. B and 0.05 p.p.m. Zn)	6.0763	7.1665	5.2561
750 p.p.m. ..	4.0878	4.5319	

the optimum boron and zinc requirements (0.3 p.p.m. of B and 0.05 p.p.m. of Zn have been shown to produce maximum dry and ash weight)—there is greater accumulation of magnesium as compared with potassium. At 0.025 p.p.m. of boron and zinc there is an increased potassium accumulation over magnesium. Thirdly, taking potassium and magnesium together in both the boron and zinc added cultures the total accumulation of these two elements is considerably greater in zinc added cultures over the boron added ones.

There is, doubtless, evidence to show that at 0.025 p.p.m. of boron or zinc a selective absorption takes place with greater tendency to accumulate potassium rather than magnesium although both the elements are available for absorption. The reverse happens, however, when 0.3 p.p.m. of boron and 0.05 p.p.m. of zinc were added to the cultures. Selective absorption



of the macro-elements perhaps takes place with variations in concentrations of the micro-elements and these results are forming the basis for further experimentation aimed at elucidating the mechanism of absorption of macro-elements by fungal cultures.

The effect of combining micro-elements on the growth of the three species of *Fusarium* was studied. In addition to Richard's medium, Czapek's, potato-dextrose and Horne and Mitters's media were also included. The concentrations of micro-elements used were as follows:—

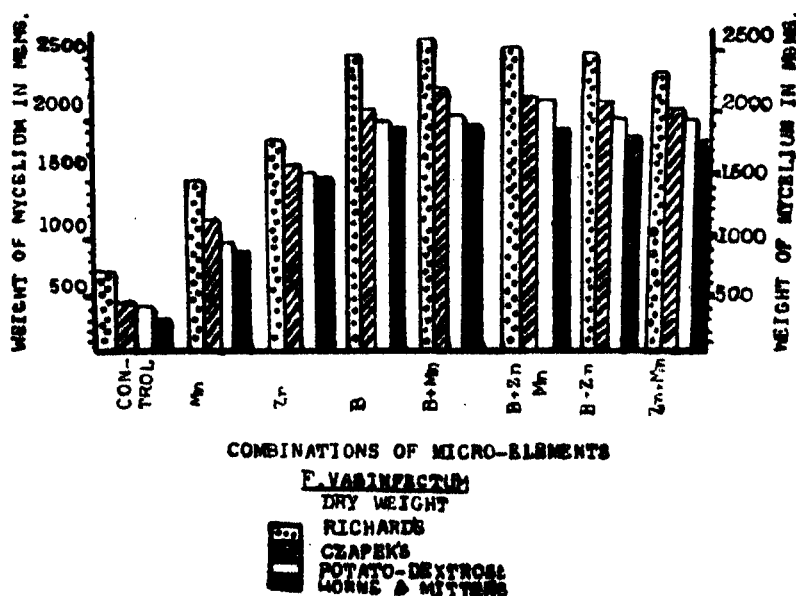
*F. vasinfectum*.—0.3 p.p.m. boron, 0.05 p.p.m. zinc, 0.2 p.p.m. manganese.

*F. udum*.—0.5 p.p.m. boron, zinc and manganese.

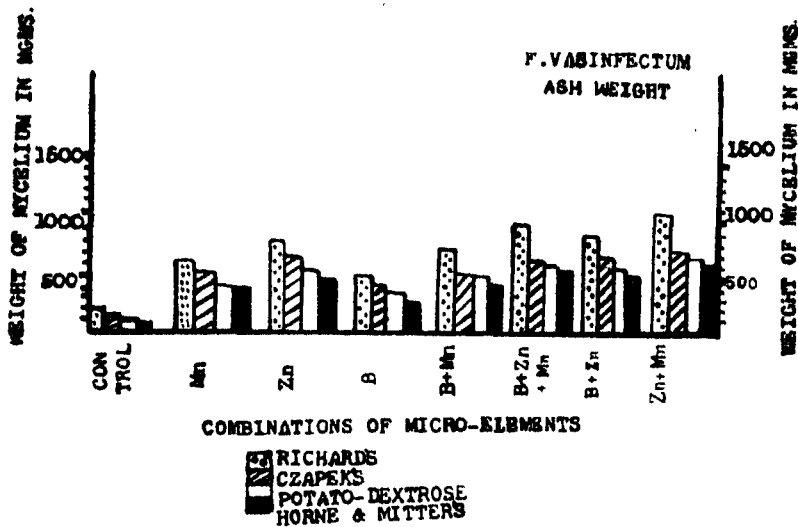
*F. moniliforme*.—0.3 p.p.m. boron, zinc and manganese.

The above figures represent the optimum levels where the micro-elements individually produced the maximum growth response for each species of fungus tested. The exact combinations in which the micro-elements were mixed with the various media and the results thereon are indicated in Text-Figs. 10 to 15.

1. It is apparent that Richard's medium gave consistently in the three fungi the highest weight (both dry and ash) followed in the decreasing order by Czapek's, potato-dextrose and Horne and Mitter's.

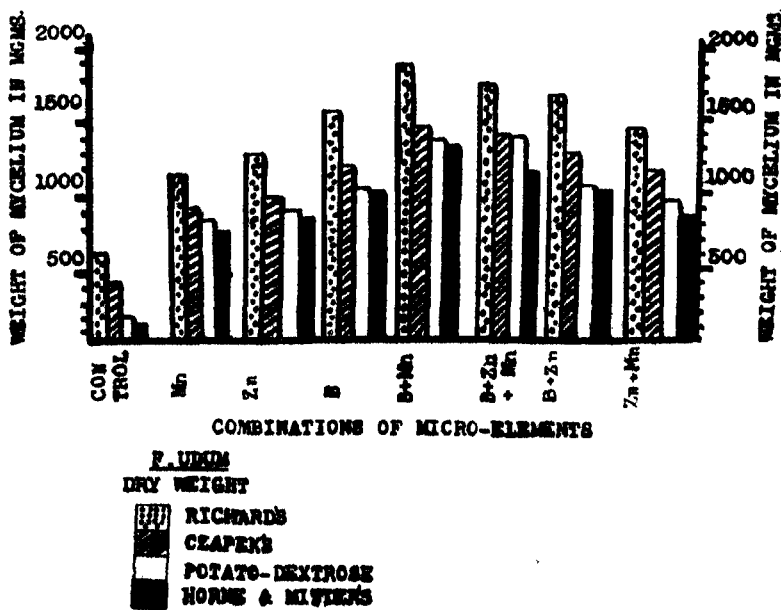


TEXT-FIG. 10.

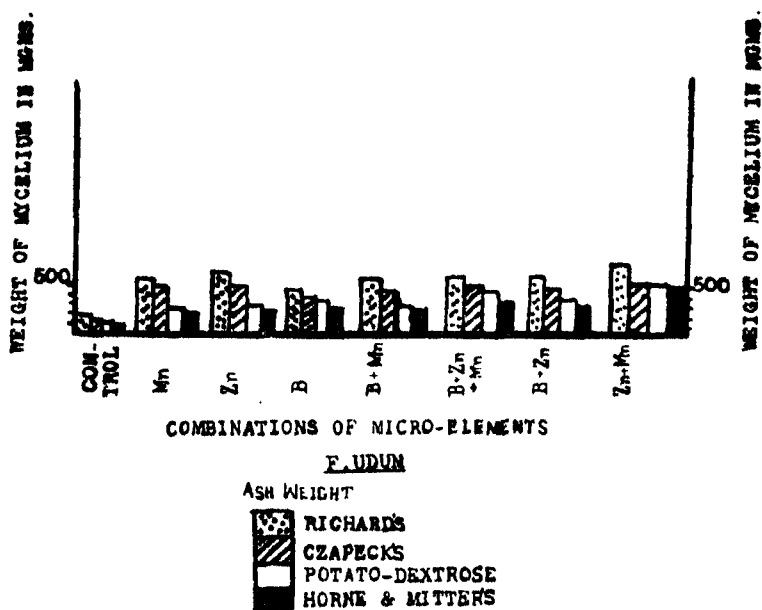


TEXT-FIG. 11.

TEXT-Figs. 10 & 11. Show the response of *F. vasinfectum* to combinations of micro-elements in four types of media.

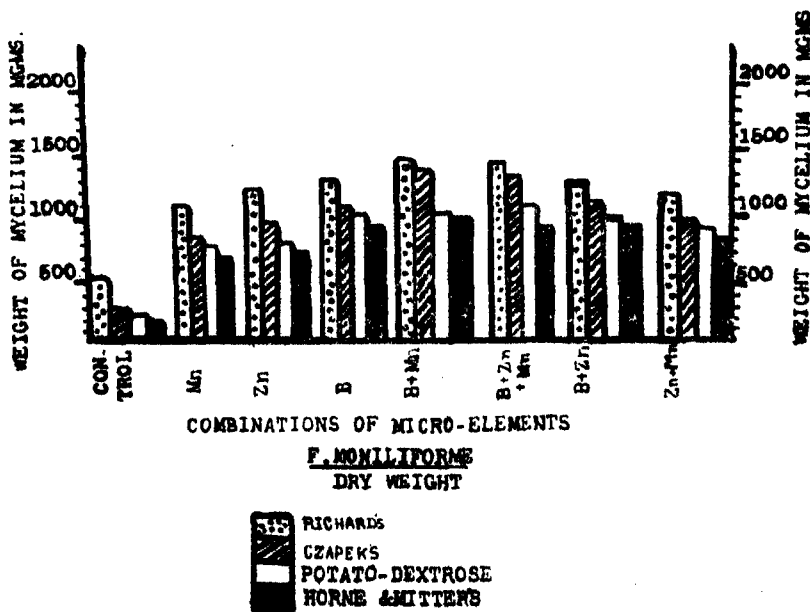


TEXT-FIG. 12.

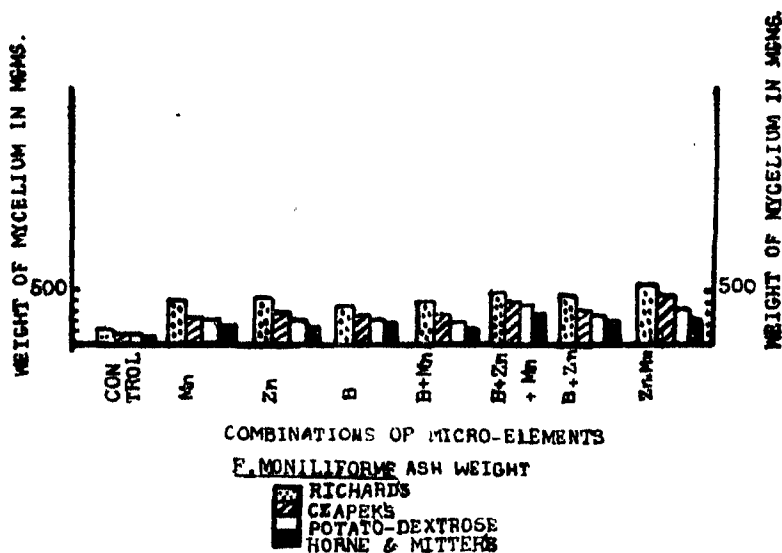


TEXT-FIG. 13.

TEXT-FIGS. 12 & 13. Show the dry and ash weights of *F. udum* with different combinations of micro-elements in four types of media.



TEXT-FIG. 14.



TEXT-FIG. 15.

TEXT-FIG. 14 & 15. Show the dry and ash weights of *F. moniliforme* grown in different combinations of micro-elements in four types of media.

2. Cultures containing boron + manganese gave peak dry weights with the three synthetic media, the combination of boron + zinc + manganese ranking second. Combinations of micro-elements gave higher dry weights than either the control or individual elements, zinc + manganese combination being an exception.

3. Potato-dextrose medium produced best dry and ash weights only in combination with all the three micro-elements.

4. Generally, micro-elements when used in combination gave higher ash weights than when used individually, exception to this being the case of boron plus manganese.

5. Peak for ash weights was seen when zinc was combined with manganese.

6. The three synthetic media in combination with the micro-elements produced comparable results in the dry and ash weights, but the organic medium (potato-dextrose) differed from them in that the highest dry and ash weights were seen in the organic medium where boron + zinc + manganese were present whereas, in the synthetic media boron + manganese produced best dry weight and zinc + manganese best ash weight.

That dry weight increased in the presence of boron was again proved by the increase seen in the higher weight shown by boron + manganese combination over manganese alone. There was a fall in dry weight whenever zinc was added to the medium; hence the low weights shown by the zinc + manganese combination.

Ash weights of the three fungi showed a different picture, viz., increase with the addition of zinc. However, zinc + manganese increased ash weight over zinc alone. In the presence of boron a fall in ash weight was noticed hence the low weight exhibited by the boron plus manganese combination. Mc Hargue and Calfee (1931) experimenting on the effect of manganese, copper and zinc on growth and metabolism of *Aspergillus flavus* and *Rhizopus nigricans* concluded that

1. Cultures of *Aspergillus* made a heavier, more rapid growth in a medium containing certain concentrations of manganese, copper, or zinc than in a medium free from these metals.

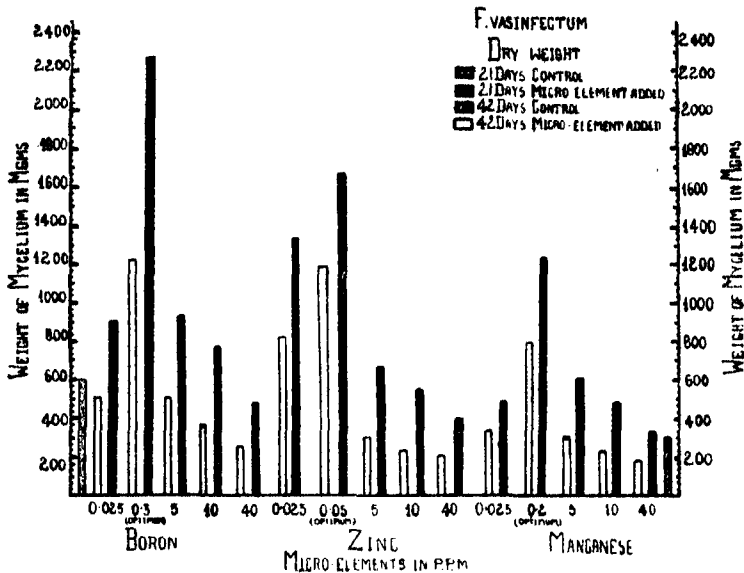
2. The optimum concentrations of these metals were low and slightly larger quantities became toxic.

3. Combinations of the optimum concentrations of manganese, copper and zinc stimulated growth greater than any one of the three metals, and all the three produced a greater growth than did the combination of any two of these elements.

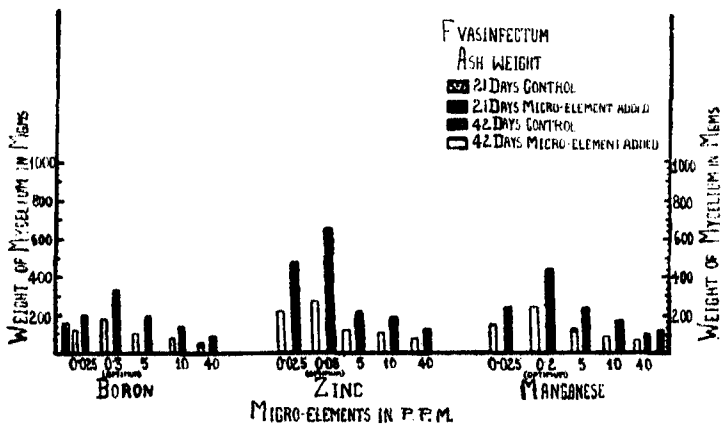
Mc Hargue and Calfee proved the importance of trace elements for the nutrition of *Aspergillus flavus* and *Rhizopus nigricans*. The present experiments not only established the importance of trace elements in the nutrition of fungi but also brought to light the selective absorption of organic and inorganic nutrients exercised by *F. vasinfectum*, *F. udum*, and *F. moniliforme*. Data collected on weights of fungal mats while determining the period of incubation required for the maximum growth of the fungus in Richard's medium showed that a lengthened period of incubation decreased the weight of the mycelium. To observe whether the results would be the same, if the experiment was repeated using media containing micro-elements, *Fusarium vasinfectum* was grown for 21 and 42 days respectively. Results are presented in Text-Figs. 16 and 17.

1. Mats taken after 21 days weighed more than the corresponding ones after 42 days (both dry and ash weight).

2. Dry weights taken after 21 days incubation in media containing boron at 40 p.p.m.; zinc at 10 to 40 p.p.m. and manganese at 0.025, 10 and 40 p.p.m. were below the weight of the control.



TEXT-FIG. 16.



TEXT-FIG. 17.

TEXT-FIGS. 16 & 17. Show the dry and ash weight production of *F. vasinfectum* after 21 and 42 days growth in the presence of micro-elements.

3. Similarly dry weights taken after 42 days incubation in media containing boron at 40 p.p.m.; zinc at 10 and 40 p.p.m. and manganese at 40 p.p.m. were below the weight of the control.

4. Ash weights after 21 days incubation in media containing boron at 10 and 40 p.p.m.; zinc at 40 p.p.m. and manganese at 40 p.p.m. were

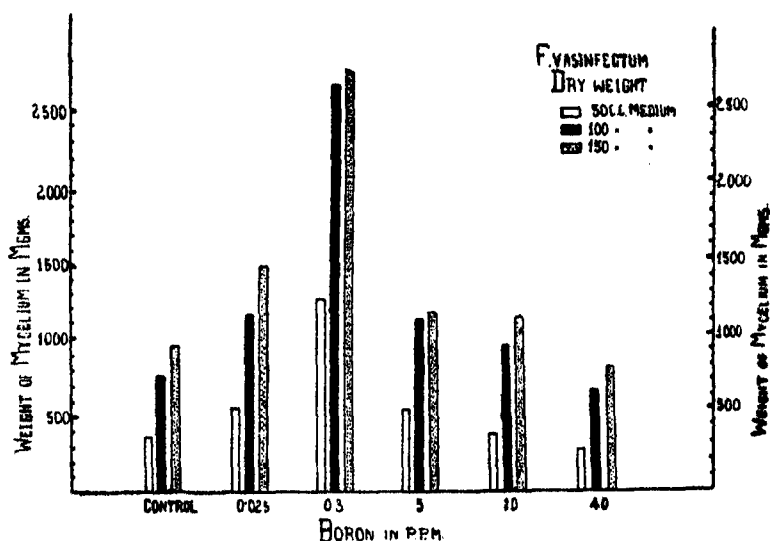
below that recorded for control. Differences in ash weight were, however, noticed in the 42-day incubated cultures as against the 21-day incubated media containing boron at 5, 10 and 40 p.p.m.; zinc at 10 and 40 p.p.m. and manganese at 10 and 40 p.p.m. were below the ash weight of the 42-day control. The fall in weights after 42 days as compared with 21-day cultures were ascribed to one of the two factors:

(a) Staling of fungal cultures or

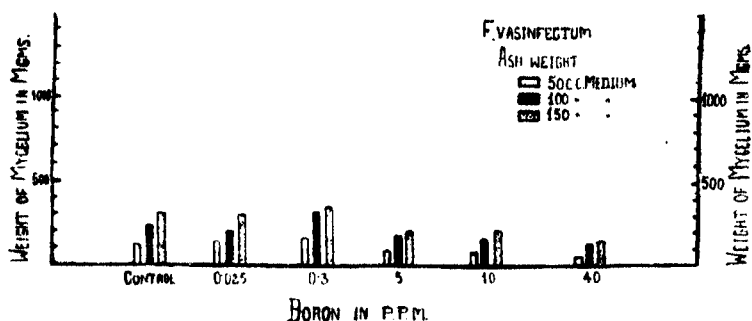
(b) Inadequate nutrient supply.

Further work was planned to gather evidence on whether either or both of the two factors mentioned above were responsible for the differences observed. The experiment consisted mainly of providing different volumes of the same medium containing micro-elements so that changes in the volume (which would indicate lower concentrations of the solutes available for fungal metabolism) would throw some light on the dry and ash weight of fungi with special reference to the total available nutriment.

The dry and ash weights of the fungus in boron, zinc and manganese were almost doubled when 100 c.c. of the medium was used instead of 50 c.c. But the increase was not kept up at the same pace when the volume of medium was increased from 100 to 150 c.c. This held good for all concentrations of micro-elements used. The increase in volume of a medium containing any element in a particular concentration increased the total weight of

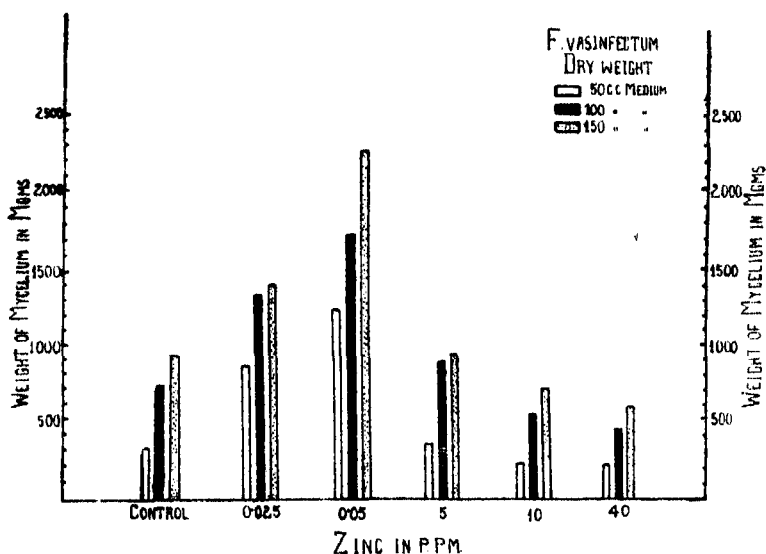


TEXT-FIG. 18.



TEXT-FIG. 19.

TEXT-FIGS. 18 & 19. Show the growth of *F. vasinfectum* in additional supply of Richard's nutrient containing different concentrations of boron.

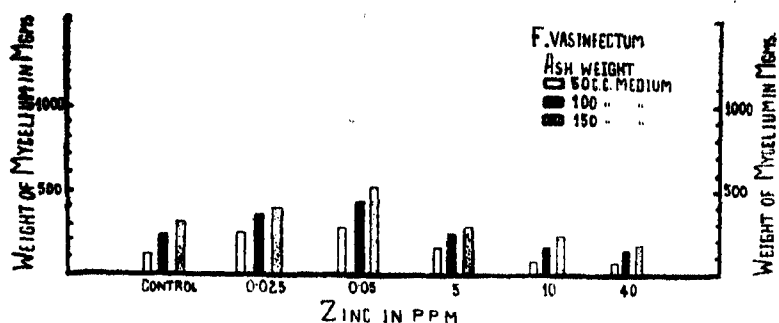


TEXT-FIG. 20.

available nutrient. But there appeared to be limitation in increase in the weight of the mat produced by increasing the total available nutriment and the indications were that the doubling of the dry and ash weights by doubling the volume of the medium was not maintained by increasing the volume any further.

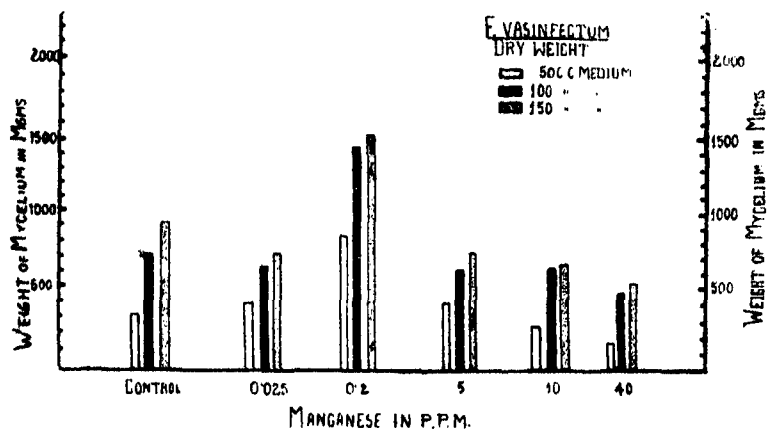
Text-Figs. 18 to 23 also show that the dry and ash weights in boron, zinc and manganese were best at 0.3 p.p.m., 0.05 p.p.m. and 0.2 p.p.m. respectively even though the volume of the medium available for fungal



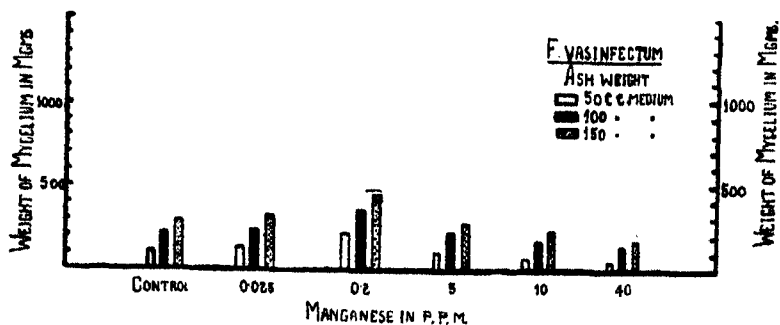


TEXT-FIG. 21.

TEXT-FIGS. 20 & 21. Show the growth of *F. vasinfectum* in additional supply of Richard's nutrient containing different concentrations of zinc.



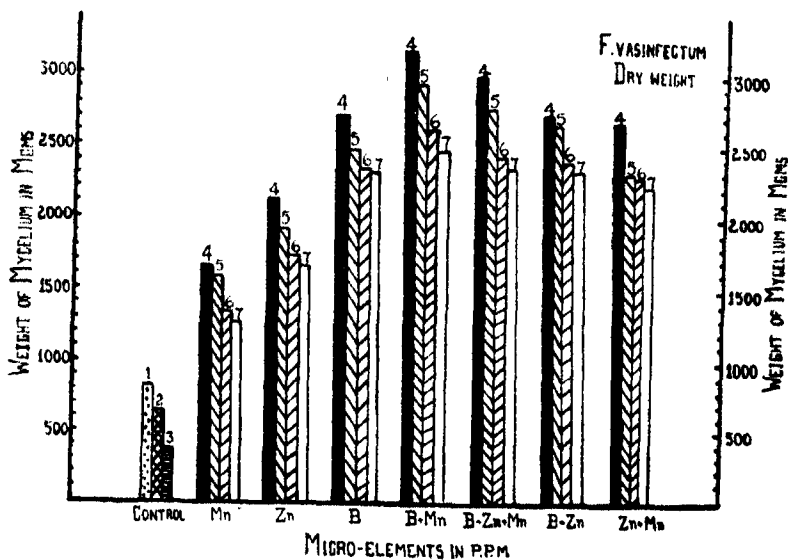
TEXT-FIG. 22.



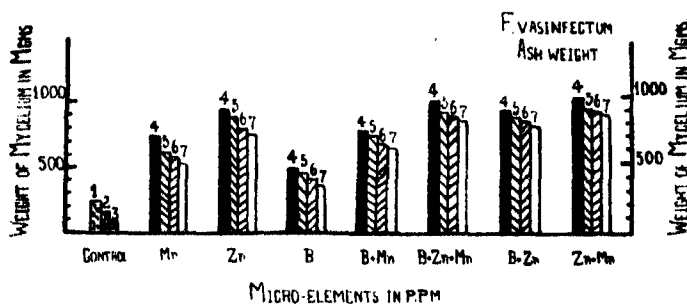
TEXT-FIG. 23.

TEXT-FIGS. 22 & 23. Show the growth of *F. vasinfectum* in additional supply of Richard's nutrient containing different concentrations of manganese.

metabolism was increased two-fold or three-fold. This increase in the volume of the medium where the element was present in a known concentration had a greater influence on weights of mat produced rather than



TEXT-FIG. 24.



TEXT-FIG. 25.

TEXT-FIGS. 24 & 25. Show the effect of replenishment of medium containing micro-elements on the growth of *F. vasinfectum*.

1. = Control. 42 days growth in Richard's medium replenished after 21 days.
2. = Control. 21 days growth in Richard's medium.
3. = Control. 42 days growth in Richard's medium not replenished after 21 days.
4. = 42 days growth in Richard's medium + micro-elements replenished after 21 days with the same medium.
5. = 42 days growth started on Richard's medium + micro-element and replenished with Richard's medium alone after 21 days.
6. = 42 days growth started on Richard's medium + micro-element and replenished after 21 days with micro-nutrient alone.
7. = 21 days growth in Richard's medium + micro-element.

the total available weight of the element concerned which naturally increased as the volume of the medium increased. The effect of replenishment of media with both macro-and micro-elements after growing the mat for 21 days was next attempted with a view to determine whether there were quantitative differences in the weights of mat produced with individual micro-elements and in combination. The results are presented in Text-Figs. 24 and 25.

The text-figs. denote the following:—

1. Weights of *F. vasinfectum* mats (dry and ash) taken after 21 days incubation in media (Richard's) containing either boron or zinc or manganese were lower than those determined after 42 days incubation having replenished the media at the end of 21 days by adding (a) Richard's nutrient solution containing either boron or zinc or manganese, (b) Richard's solution alone and (c) the three micro-nutrients alone.

2. The dry and ash weights were higher when *F. vasinfectum* was grown in Richard's nutrient solution containing either of the three micro-nutrient elements and replenished with a similar solution, after 21 days than when the medium was replenished with Richard's macro-nutrient alone or micro-nutrient (B, Zn, Mn; B plus Mn; B plus Zn; Zn plus Mn; B plus Zn plus Mn).

3. Dry weight taken after 42 days incubation was higher in media containing boron at 0.3 p.p.m. plus manganese at 0.2 p.p.m. and replenished with the same solution at the end of 21 days incubation than when media containing boron plus manganese plus zinc were used both at the start and for replenishment. However, ash weight of the fungus after 42 days incubation was higher when media containing zinc at 0.05 p.p.m. plus manganese at 0.2 p.p.m. were replenished with similar solution after 21 days incubation than when media containing boron plus manganese plus zinc were used at the start and for replenishment.

4. Weights of fungal mats after 21 and 42 days' incubation in basic media (Richard's macro-nutrient solution) were lower than those obtained after 42 days' incubation in similar media having replenished them with the same media at the end of 21 days.

5. Cultures with micro-nutrients when present either singly or in combination gave higher weights over those with macro-nutrient alone.

6. Replenishment of the media with Richard's macro-nutrient containing micro-nutrient (either singly or in combination) was more beneficial

than replenishing with either the macro-nutrient or the micro-nutrient separately.

Boyle (1924) working on the growth reactions of certain fungi to their staling products came to the conclusion that exhaustion of food material by growing a fungus in a liquid medium was not the only cause for inhibiting spore germination in the exhausted medium. He grew in sterile apple extract, a species of *Fusarium*; filtered off the staled liquid and added various proportions of fresh apple extract as well as sterile distilled water. In those he germinated *Botrytis cinerea* and concluded that dilution of the staled medium with apple extract or water increased the percentage germination with increased dilution. In other words he viewed that inhibition of fungal growth was more due to the toxic effect of the stale product rather than depletion of nutriment. Although the present work is not strictly comparable with Boyle's the fundamental objection to Boyle's technique is that he used staled products of one genus on the power of germination of another in the presence of that stale product. It must be mentioned that the work of Boyle is not based on present knowledge on the development of studies on "antibiotics" where rapid advances have been made to show that the products of metabolism of one organism have a retarding effect on the growth of another. In the present case enrichment of the substratum after production of stale products following incubation has shown that the same organism regenerates better (as judged by dry and ash weight production of mats) when fresh nutrient solution is added to the staled one consisting of (a) macro-nutrient elements alone, (b) the macro-nutrient element plus the micro-nutrient element and (c) micro-nutrient element alone. It should, however, be stated that the effect of the stale products produced in the case of *F. vasinfectum* may not act as an inhibitor on its own growth as effectively as those observed by other authors. Nevertheless, the fact remains that the growth (dry weight and ash weight production) of *F. vasinfectum* increases with increasing doses of nutrition either received in the beginning or given at a later stage of growth by way of replenishment after the formation of stale products.

#### DISCUSSION

Steinberg (1942) after an exhaustive work on the nutrition of *Aspergillus niger* concluded that trace-elements probably played a specific part in the utilization of CO<sub>2</sub> by the fungus. Zinc was considered to help in vital oxidation and reduction and also acted adversely upon the accumulation of organic acids in fungal cultures (Foster, 1939). That these findings have bearing on fungal taxonomy (which has hitherto been based largely on

morphological characters alone) and also on the modes of perennation of fungal saprophytes and facultative parasites in the soil is becoming increasingly apparent.

The difference in behaviour of boron and zinc with regard to the dry and ash weight production in fungal growth has not been mentioned so far by previous workers. This could easily be explained by the fact that the investigations were confined to dry weight determinations of fungal mats alone. The interesting data recorded here is an addition to the present-day knowledge of fungal nutrition.

A start has been made for quantitative analysis of ash of boron and zinc cultures by using standard methods of analysis results of which are mentioned in Tables I and II. The present indications are that potassium and magnesium salts get accumulated to a greater extent in zinc added cultures over that of boron resulting in an increased ash weight. Whether the accumulation of the macro-nutrient salts varies with the decreased or increased presence of trace element remains to be worked out. Nevertheless, the results clearly denote the tendency for increased accumulation, generally speaking, of inorganic salts in fungal mats to a greater extent in zinc added cultures than in boron added ones. There are also indications of a selective absorption of the macro-elements in the presence of zinc or boron at certain concentrations.

The results of the experiments on the response of the three fungi to combinations of micro-elements confirmed the fact that in the combination boron plus manganese or boron plus zinc or all three elements together (B plus Mn, B plus Zn, B plus Mn plus Zn) the dry weight was better than in boron alone or the combination Mn plus Zn. Of the three boron combinations, B plus Mn, gave the best dry weight. Similarly, high ash weights were recorded when zinc was used in three combinations with manganese and boron (Zn plus Mn; Zn plus B; Zn plus Mn plus B). It may be recorded, however, that Zn plus Mn combination gave the heaviest ash-weight.

Diminished growth of the fungus estimated on a quantitative basis (both dry and ash weight) after prolonged growth in the same medium appeared to be connected with the lack of adequate supply of nutrients. The dry and ash weight output of the mat, however, increased with increase of total available solutes. Further, it was observed that replenishment of fungal cultures after the formation of stale products helped the fungal growth, and such replenishments when done with the macro-nutrient solution plus

either boron, zinc or manganese was better than when macro-nutrient alone was added.

A number of questions arise on the role of trace-elements in fungal nutrition. Are the trace elements accumulated by the fungus; if they are accumulated what is the quantity involved? What fraction of the element supplied originally is taken up by the fungus? Is there a selective absorption of the individual trace element when present in combination with other elements? What is the relationship between the absorption and accumulation, if any, of boron, zinc and manganese?

Detailed quantitative analyses of the ash both chemically and spectroscopically now underway, may resolve some of these difficult problems.

#### SUMMARY

A number of experiments were performed to study the nutritional physiology of *Fusarium vasinfectum*, *F. udum* and *F. moniliforme*. The results are summarised as follows:

1. *F. vasinfectum*, *F. udum* and *F. moniliforme* are physiologically alike.
2. The three strains favoured media with a high C/N ratio (Richard's) as judged by dry and ash weights and they required three weeks incubation for maximum growth.
3. Growth of the three species was good over a wide range of pH values, optimum being at pH 5.
4. *F. vasinfectum*, *F. udum* and *F. moniliforme* responded well to various concentrations of boron, zinc and manganese. Optimum levels were low and differed with the three strains. (*F. vasinfectum* = 0.3 p.p.m. of B; 0.05 p.p.m. of Zn; 0.2 p.p.m. of Mn. *F. udum* = 0.5 p.p.m. of B, Zn and Mn. *F. moniliforme* = 0.3 p.p.m. of B, Zn and Mn). Higher concentrations were not toxic. All the three strains yielded heaviest dry weights in nutrient media containing boron but highest ash weight was recorded in nutrient media containing zinc. This important fact was noticed in every one of the experiments when comparisons between zinc and boron were undertaken.
5. Combination of micro-elements (B, Mn and Zn) showed better weights than individual elements. B plus Mn combination proved the best for dry weights while Zn plus Mn was the best for ash weights for the three fungi.
6. Dry and ash weights of *F. vasinfectum* fungal mats after 21 days growth were more than those after 42 days.

7. Weight (dry and ash) of *F. vasinfectum* mats increased with increase in volume of Richard's medium.

8. Replenishment of fungal cultures (*F. vasinfectum*) with Richard's medium after the formation of stale products increased the dry and ash weight of the mats. Similar replenishments with micro-elements + Richard's medium after 21 days growth produced increase in dry and ash weight of the mats over cultures where Richard's medium or micro-element solutions alone were used for replenishment. Details of these are given in the text.

#### ACKNOWLEDGEMENTS

The author is greatly indebted to Prof. T. S. Sadasivan, M.Sc., Ph.D. (London), Director, University Botanical Research Laboratory, Madras, for suggesting the problem and offering ready guidance and helpful criticism throughout. Thanks are due to the authorities of the University of Madras for the award of a Studentship during the tenure of which part of this work was undertaken and to the Government Mycologist, Coimbatore, for the supply of fungal cultures.

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# THE EFFECTS OF POTASSIUM DEFICIENCY ON RICE

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## INTRODUCTION

ALTHOUGH potassium exists in plants almost entirely in ionic form it is known to be involved in several biochemical reactions within the plant, the exact role of potassium is, however, obscure. It has long been accepted that potassium is essential for photosynthesis, for conversion of sugars into starch, and for translocation of carbohydrates within the plant. The dependence of protein synthesis, particularly in meristems, on an adequate supply of potassium is also well established. In the absence of potassium normal cell division does not take place. Besides influencing these two groups of reactions potassium serves as a buffering agent and facilitates the action of various enzymes in the plant. Respiration, permeability of cytoplasmic membranes, as well as hydration of protoplasm are also controlled by potassium. The absorption and movement of certain other mineral elements have been shown to depend on potassium supply. Thus the movement of iron is restricted in cases of potassium deficiency so that chlorophyll formation is interfered with. The efficiency of potassium is particularly noteworthy under restricted conditions of light. Potassium possesses feeble radioactivity but there is no convincing evidence that this property has any beneficial action on the growth of plants.

From a practical standpoint an adequate supply of potassium serves to increase root development, to strengthen the straw and to increase the plumpness of grain in cereals, and generally to improve the power of the plant to retain water and to improve the general vigour of the plant which thereby becomes disease-resistant.

Potassium deficiency causes serious morphological symptoms in most plants. It is generally held that tillering in cereals is not prevented. The quality of several important economic crops is however seriously affected. Thus the appearance and burning quality of tobacco, the cooking quality

of potatoes, the milling quality of barley, the sucrose content of sugar beet, the purity of sugarcane juice, and the chunkiness of sweet potatoes are all adversely affected by potassium deficiency.

A full discussion of the effects of potassium deficiency on plants may be found in the monograph by Eckstein, Bruno and Turrentine (1937). A comprehensive summary of recent work on potassium deficiency symptoms has been published by Hoffer (1938).

From the vast literature on potassium deficiency one may assume that an investigation of its effects on rice is likely to be of economic importance. It is generally recognized that an average rice crop removes about 40 pounds or more of potash per acre and that the potash content of the crop is equal to or higher than the nitrogen content of the crop. It is therefore surprising that there have been few reports of the response of rice to potash. Sethi (1943) has pointed out that under Indian conditions the need for supplying potash as a manure for rice is comparatively unimportant.

Owing to the poor response to potash in rice manuring, the effects of potassium deficiency on rice do not appear to have been properly investigated. The references to this subject in the published literature are very few and limited in scope.

Kellner *et al.* (1890) found a low response to potash in a Japanese rice soil. There was no effect on tillering. Manuring the soil with potash together with an adequate application of nitrogen and phosphoric acid produced a small increase in yield of rice.

Gericke (1930) examined the effects of various nutrient elements on rice in solution culture and found that potash was required by the plant throughout its life period under the conditions of the experiment. Ishizuka (1934) confirmed this result in sand culture.

Carberry (1933) studied the composition of rice grown in sand culture with nutrient solutions deficient in potash and found that the percentage of potash in the crop was much lower than that found in the crop grown with the complete nutrient solution. He did not report any other abnormality.

Schropp (1934) made a comparative study of the effects of potassium deficiency on rice and other cereals in sand culture. He found that the young rice leaves had a blue green colour while the older leaves had numerous brown spots and that grain formation was reduced.

Finally, in an investigation of the cause of "mentek" disease of rice in Java, Kuilman (1936) carried out solution culture and field studies with rice.

Under potassium deficiency he found that the older leaves of the plant became yellow as with nitrogen or phosphate deficiency but the most characteristic effect found by him was a shortening of the younger leaves. The symptoms of "mentek" disease observed in the field were found to be similar to those caused by potassium deficiency in sand culture.

On the whole, it appears that most of the existing information on the effects of potassium deficiency on rice has been derived from solution cultures and sand cultures.

The work to be described here was carried out in connection with the investigation of the causes of a disease of rice prevalent in areas near Mandalay in Upper Burma. This disease is known among local cultivators under the name "amyitpo" which literally means "root disease". The soil in which the disease occurred was found to cause symptoms of potassium deficiency in rice and to respond to potassium manuring. The data obtained in manurial experiments with the soil in pot culture to study the effects of potassium deficiency on rice are dealt with in the present article. The results obtained in the study of "amyitpo" disease will be dealt with in a separate article.

#### METHODS AND MATERIALS

Standard methods were used in the analysis of soil and plant material except where otherwise indicated. (A.O.A.C., 1937; Wright, 1937, 1938).

Pot culture experiments were carried out with soil from a cultivator's field at Zigyogon village, Patheingyi township, near Mandalay. The soil will be referred to as Patheingyi soil. Rice grown in the soil exhibited striking symptoms which were shown to be caused by potassium deficiency by chemical analysis and confirmed by the response to manuring and by observations in solution culture.

Patheingyi soil is a heavy clay of greyish black colour developed from material of dolomitic origin. Large boulders of rock were present in the fields, while calcareous nodules were abundant in the soil. The soil data are shown in Table I.

The analytical data of Table I indicate that Patheingyi soil is well provided with all the nutrient elements. There is thus no clue available to predict a response to any given treatment. Attention may, however, be directed to the relatively high percentage of available phosphoric acid, the fairly wide carbon-nitrogen ratio, and the abnormally high proportion of exchangeable magnesium compared to calcium.

TABLE I

*Composition of Patheingyi soil sampled for pot culture work*

Items	Percentages on oven dry soil
<i>Mechanical analysis—</i>	
Coarse sand ..	2.3
Fine sand ..	12.2
Silt ..	27.0
Clay ..	55.8
Calcium carbonate ..	3.2
<i>Composition of concentrated HCl extract—</i>	
CaO ..	2.26
MgO ..	1.18
K <sub>2</sub> O ..	0.96
P <sub>2</sub> O <sub>5</sub> ..	0.11
<i>Other data—</i>	
Available K <sub>2</sub> O (Dyer) ..	0.035
Available P <sub>2</sub> O <sub>5</sub> ..	0.042
Organic N ..	0.070
Organic C ..	1.250
Carbon—Nitrogen ratio ..	18:1
pH ..	8.15
<i>Exchangeable bases in 100 g. of air dry soil:</i>	
Ca Milreqts ..	14.20
Mg do ..	30.20
K do ..	2.02
Na do ..	1.45

## EXPERIMENTAL DATA

*(a) Pot culture experiment with Patheingyi soil*

Seedlings of Taungdeikpan variety of rice were raised in unmanured surface soil and after five weeks' growth they were transplanted on 3rd August, 1940, each pot receiving one seedling. Manures were applied on 14th August 1940 at the rate of 80 lbs. per acre of N, P<sub>2</sub>O<sub>5</sub> or K<sub>2</sub>O in the form of ammonium sulphate, orthophosphoric acid, and potassium sulphate respectively. There were eight treatments consisting of O, N, P, K, NK, PK, NP, and NPK, each treatment being replicated four times. Further details of management of the pots may be found in a previous publication by the author (Aiyar, 1946). The rates of application of manures have been kept high so as to overcome inherent possibilities such as microbial absorption, inorganic fixation, ion antagonism and to leave an adequate surplus for the crop. Economic considerations have not been taken into account as the problem is dealt with exclusively from the chemical standpoint.

During the growth period various observations were made from time to time. From Plate III (a), (b), it may be seen that rice responded in

Patheingyi soil to potash and to a lesser extent to nitrogen but not to phosphoric acid. The response to potash was evident within two weeks after manuring. The data on plant measurements and tillering are shown in Table II.

TABLE II  
*Characteristics of rice plants grown in Patheingyi soil*  
(Mean values of observations)

Height of plant cm.				Length of leaf cm.		Maximum width of leaf mm.		Index of leaf area sq. cm	Tillers per pot		Panicles per pot	
Treatment	15-9-40	15-10-40	15-11-40	15-9-40	15-10-40	15-9-40	15-10-40	15-9-40	15-9-40	15-10-40	15-11-40	15-11-40
O	44	58	71	26.8	29.5	4	5	11.0	4	4	9	2
N	60	83	116	35.3	44.6	6	7	19.3	4	14	13	13
P	63	74	80	36.6	42.5	6	7	18.1	4	6	12	4
K	88	90	125	43.3	54.4	8	9	34.6	8	16	12	12
NK	76	94	122	38.2	58.9	7	9	26.4	6	15	13	13
PK	74	92	114	34.0	55.4	7	9	20.7	5	10	10	10
NP	69	85	117	30.7	49.6	6	7	16.7	4	11	11	10
NPK	69	89	121	33.4	52.2	7	8	22.8	5	16	15	15

From the data of Table II it may be conclusively stated that the main response is obtained with potash treatment which is evident from the earliest date of observation. It is interesting to note, however, that although nitrogen does not show any appreciable response in the beginning it soon catches up and ultimately the response becomes equal to that given by potash. Another striking result revealed by Table II is the fact that in the absence of potash or nitrogen tiller formation is slow in the beginning but increases rapidly just before flowering though the late tillers are unfruitful as may be judged by the small number of panicles in treatments O and P. The influence of treatment on certain plant characters and yield is shown graphically in Fig. 1.

A peculiar characteristic observed in this experiment was a shortening of the leaf-blades and internodes in the absence of potash. Some illustrative data are given in Table III and exhibited graphically in Fig. 2.

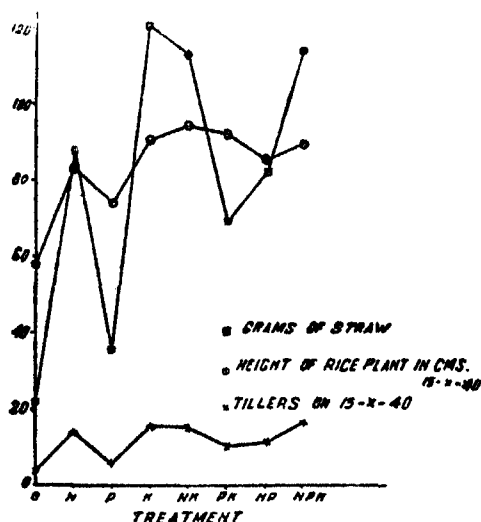


FIG. 1

FIG. 1. Response of rice to potash treatment in Patheingyi soil.

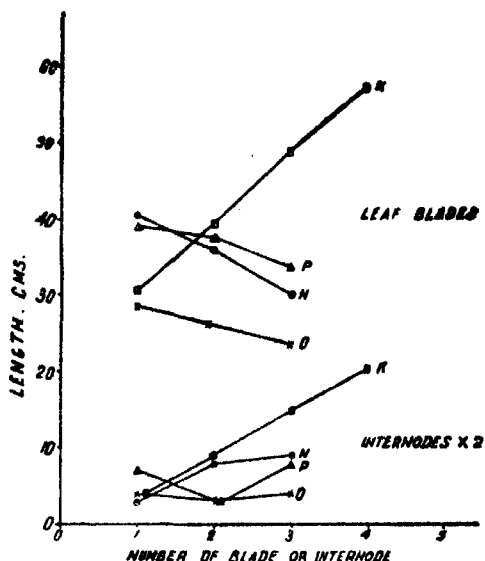


FIG. 2

FIG. 2. Effect of potassium deficiency on the length of leaf-blades and internodes of rice.

TABLE III

Variation in length of leaf-blades and internodes of rice plants grown in Patheingyi soil

(15 - IX - 40)

Items	Treatment			
	O	N	P	K
Blade No.	cm.	cm.	cm.	cm.
1 (Oldest)	28.5	40.5	39.0	50.5
2	26.5	36.0	37.5	39.5
3	23.5	30.0	33.5	49.0
4	..	..	..	57.0
Internodes	cm.	cm.	cm.	cm.
1 (Oldest)	2	1.5	3.5	2
2	1.5	4	2	4
3	2	4.5	4	7.5
4	..	..	..	10.5

In addition to various measurements recorded in Tables II and III a careful record was kept of the appearance of plants from time to time. A description of the variously treated rice plants is given in Table IV,

TABLE IV

*Appearance of rice plants grown in Patheingyi soil*

Treatment	Observations on 15-10-40
O ..	Plants stunted and bushy with dark blue green colour. Stems short and thick. Leaves short, stiff and erect or spread out at a wide angle (almost horizontal). Curvature restricted to the end of the leaves and shaped like a hook in the case of erect leaves or turned upwards when the leaves are horizontal. High proportion of leaf drying from the oldest leaf upwards. No trace of yellow colour during drying. Chocolate coloured patches on leaf surface. Rapid decay of dried leaves. Leaf-sheaths dried white.
N ..	Tall plants with dark green colour. Broad, dark green leaves with chocolate patches though rather less than in control. Leaves drying from edges inward without a trace of yellow colour. Erect and horizontal habit in leaves. Leaf-sheaths drying to white.
P ..	Stunted plants similar to control but taller and darker green.
K	Tall healthy plants with normal yellowish green colour. Leaves showed no stiffness or tendency to remain erect or horizontal. Curvature normal. No chocolate colour or abnormal drying. Leaves occasionally dried from tips downward but accompanied by yellowing.
NK ..	Similar to K in all respects.
PK	Plants somewhat stunted with some stiffness and horizontal habit in leaves. Drying as in control. Distinctly worse than K.
NP ..	Dark green plants similar to N.
NPK ..	Plants equal in size to K or NK treated plants. Leaf yellowing present though slight. In other respects similar to NP.

From a consideration of the data presented in Tables II, III and IV it may be concluded that rice plants grown in Patheingyi soil are affected by potassium deficiency and exhibit characteristic symptoms which can be corrected by treatment with potash.

It may be added further that out of all the eight treatments the control and P treated plants only remained green and immature when examined on 16-12-40, the date of harvest. In all the other treatments the plants received either potash or nitrogen which seems to have helped the plant to acquire potash from the soil.

The yield data are presented in Table V.

TABLE V

*Yield of straw and grain from rice plants grown in Patheingyi soil*

Treatment	Straw : grams per pot				Total straw grams	Grain: grams per pot				Total grain grams
	A	B	C	D		A	B	C	D	
O ..	4.5	5.7	6.5	4.8	21.5	2.2	0.5	1.2	0.8	4.7
N ..	24.6	18.7	22.9	21.9	88.1	20.3	18.0	21.7	19.3	79.3
P ..	6.9	6.4	10.0	10.0	35.3	1.2	4.4	5.6	4.5	15.7
K ..	29.8	30.6	29.0	30.7	120.1	21.9	21.6	21.0	21.8	85.3
NK ..	28.5	28.3	28.4	27.2	112.4	20.4	21.8	22.5	17.5	82.2
PK ..	20.2	15.0	11.3	22.3	68.8	18.5	17.5	13.5	20.0	69.5
NP ..	17.7	19.1	22.1	22.7	81.6	21.5	22.0	22.0	25.0	90.5
NPK	33.8	23.0	30.6	28.6	114.0	28.0	24.2	29.5	26.5	108.2

	Straw	Grain
Standard error of each total yield .. .. .	5.58	3.85
Critical difference between two total yields for significance at 1% level. .. .. .	22.36	15.41

It may be seen from the yield data presented in Table V that high yields are associated with potash treatment while phosphoric acid treatment produces a depressing action (Fig. III). When nitrogen is present the effect of potassium deficiency is largely overcome and a high grain yield is produced. This beneficial effect of nitrogen is however exerted rather slowly as it has not enabled the plant to produce more than a moderate yield of straw.

The interactions  $N \times K$  and  $K \times P$  were negative but not significant in the case of straw. The analysis is shown in Tables VI and VII respectively.

TABLE VI

*Interaction between N and K*

		Straw		
		No N	With N	Total
No K ..	..	56.8	169.7	226.5
With K ..	..	188.9	226.4	415.3
Total ..	..	245.7	396.1	
Difference (mean) ..	..	..	- 4.7	

Critical difference for significance at 1% level. .. .. . 7.92



TABLE VII

*Interaction between P and K*

		Straw		
		No P	With P	Total
No K	..	109.6	116.9	226.5
With K	..	232.5	182.8	415.3
Total	..	342.1	299.7	
Difference (mean)	..		- 3.6	

Critical difference for significance at 1% level .. .. . 7.92

After harvest the crop produced in the pots was dried, weighed and then analysed. The composition of the straw and grain is shown in Table VIII.

TABLE VIII

*Composition of rice plants grown in Patheingyi soil*

(Percentages on oven dry basis)

Treatment		Straw							Grain		
		N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	CaO	MgO	F <sub>2</sub> O <sub>3</sub>	Mn	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
O	..	1.512	0.320	1.304	0.265	0.608	0.204	0.016	1.771	0.724	0.248
N	..	0.371	0.084	2.723	0.198	0.288	0.068	0.009	0.959	0.598	0.317
P	..	0.679	0.250	1.540	0.255	0.400	0.158	0.012	1.463	0.698	0.238
K	..	0.322	0.095	2.818	0.191	0.336	0.024	0.018	0.945	0.611	0.363
NK	..	0.317	0.105	2.418	0.213	0.352	0.029	0.016	0.931	0.582	0.370
PK	..	0.473	0.121	2.367	0.213	0.368	0.038	0.022	1.015	0.631	0.321
NP	..	0.443	0.085	2.785	0.246	0.400	0.052	0.014	1.001	0.648	0.303
NPK	..	0.329	0.076	2.877	0.213	0.344	0.029	0.012	1.043	0.603	0.375

The data of Table VIII reveal several interesting features: (1) In the absence of potash the plant contains abnormally low percentages of this nutrient but when manured with potash or even nitrogen the potassium content rises to a high level (Fig. 3) and the yield is increased in proportion to the potash absorbed by the crop (Fig. 4). (2) The nitrogen content of straw as well as of grain is abnormally high when potash is not used in the manure but the application of potash or nitrogen brings down the nitrogen percentage to the normal level according to the percentage of potassium in

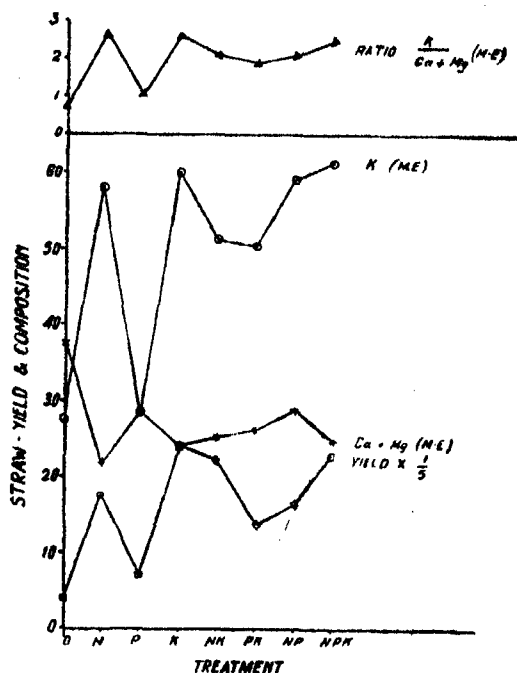


FIG. 3

FIG. 3. Effect of potassium on yield and composition of rice straw grown in Patheingyi soil.

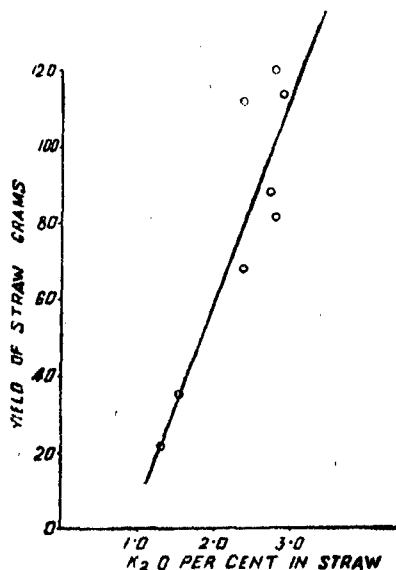


FIG. 4

FIG. 4. Relation between potassium content and yield of rice straw grown in Patheingyi soil.

the plant (Fig. 5). (3) There is a tendency for the accumulation of phosphoric acid in the straw as well as grain unless sufficient potash is absorbed by the crop (Fig. 6). (4) As in other well-known cases of potassium

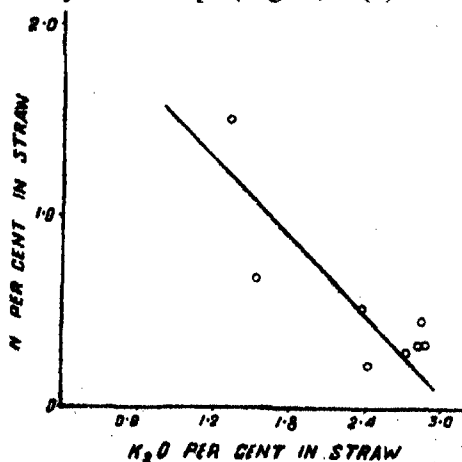


FIG. 5

FIG. 5. Inverse relation between nitrogen and potash contents of rice straw grown in Patheingyi soil.

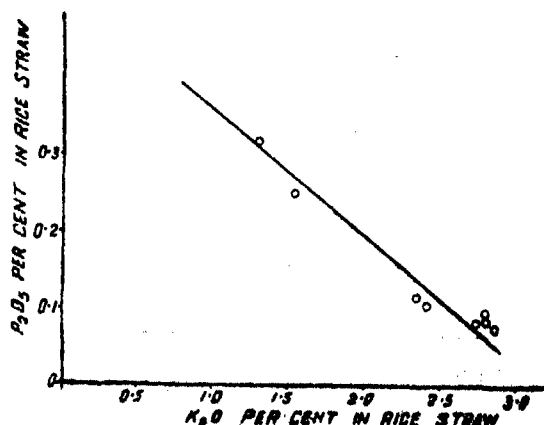


FIG. 6

FIG. 6. Inverse relation between phosphoric acid and potash contents of rice straw grown in Patheingyi soil.

deficiency the rice plant shows an increased absorption of calcium and magnesium which is in inverse relation to the potassium absorbed by the crop (Fig. 7). (5) Finally there is a very definite increase in the iron content of straw in the absence of potash and the inverse relation between potassium content and iron absorbed is shown in Fig. 8.

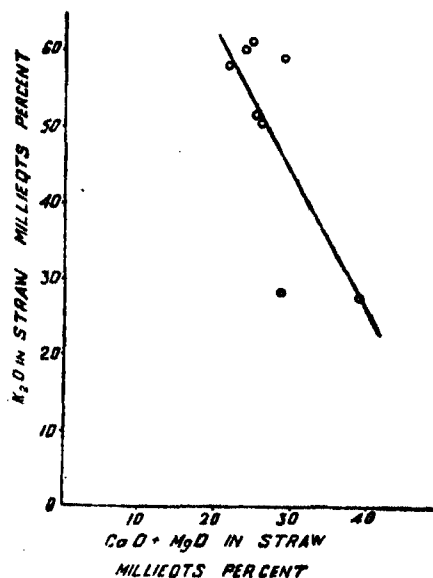


FIG. 7

FIG. 7. Calcium and magnesium in relation to potassium absorbed by rice straw grown in Patheingyi soil.

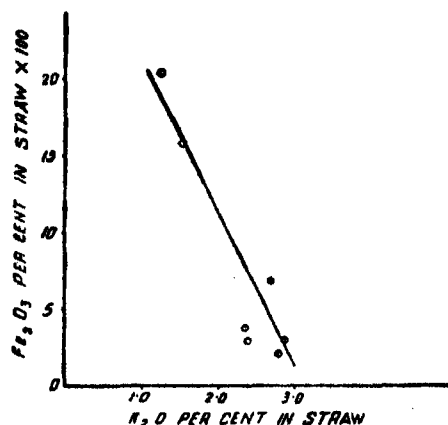


FIG. 8

FIG. 8. Accumulation of iron in rice straw grown under potassium deficiency in Patheingyi soil.

The peculiarities in the composition of the rice plant grown in Patheingyi soil suggest some close interrelationships which are presented in Table IX.

TABLE IX  
*Correlation between plant characters and composition of rice plants grown in Patheingyi soil*

Related quantities		Correlation coefficient		Calculated value of <i>t</i>	Level of significance <i>P</i>
X	Y	<i>r</i>	Standard error		
K <sub>2</sub> O% in straw	Height of plant on 15-10-40	+ .8187	± .2345	3.49	3%
do	Yield of straw	+ .8960	± .1796	5.00	1%
do	Yield of grain	+ .7931	± .2476	3.21	2%
do	N% in straw	- .8243	± .2235	3.51	2%
do	P <sub>2</sub> O <sub>5</sub> % in straw	- .9272	± .1529	6.06	1%
do (m.e.)	CaO + MgO% in straw (m.e.)	- .7500	± .2700	2.78	5%
N% in straw	Yield of straw	- .9782	± .0648	11.52	1%
do	P <sub>2</sub> O <sub>5</sub> % in straw	+ .9921	± .0509	19.29	1%

*(b) Experiments in culture solution*

The composition of the nutrient solution used in this work was based on that of Kuilman (1936) but some important modifications were made. The pH of the complete nutrient solution was 5.0 and all other solutions were adjusted to pH 5.0 by the addition of dilute solutions of sulphuric acid or caustic soda as required. The solution had an apparent osmotic pressure of 0.52 atm. The concentrations of the nutrient solutions are shown in Table X.

TABLE X

*Composition of nutrient solutions used in the study of the effects of potassium deficiency on rice*

Salts used	Concentration of salt in mg. per litre				Elements supplied
	Complete Full K	1/10 K	1/100 K	Minus K	
NH <sub>4</sub> NO <sub>3</sub> ..	340	340	340	340	N
KNO <sub>3</sub> ..	170	17	1.7	..	N, K
KCl ..	300	30	3.0	..	K
CaCl <sub>2</sub> ..	25	25	25	25	Ca
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O ..	50	50	50	50	Ca, P
MgSO <sub>4</sub> ·7H <sub>2</sub> O ..	250	250	250	250	Mg, S.
NaNO <sub>3</sub> ..	..	126	140	140	N
NaCl ..	..	216	240	240	..
Ferric citrate scales ..	54	54	54	54	Fe
MnCl <sub>2</sub> ·4H <sub>2</sub> O ..	36	36	36	36	Mn
H <sub>3</sub> BO <sub>3</sub> ..	1.1	1.1	1.1	1.1	B
ZnSO <sub>4</sub> ·7H <sub>2</sub> O ..	0.9	0.9	0.9	0.9	Zn
CuSO <sub>4</sub> ·5H <sub>2</sub> O ..	0.2	0.2	0.2	0.2	Cu
K m.e. per litre ..	5.76	0.58	0.06	..	
Ca + Mg ..	2.94	2.94	2.94	2.94	
Na ..	..	5.2	5.7	5.8	
K : Ca + Mg ratio ..	2 : 1	0.2 : 1	0.02 : 1	..	

Each treatment was replicated four times in wide-mouthed bottles of two litres capacity. The bottles were carefully blackened by pasting light proof black paper. Seeds of Taungdeikpan variety of rice were sown in clean sand and after 18 days' growth the seedlings were transferred to the appropriate culture solution each bottle receiving one seedling. Solutions were renewed once a week to provide aeration and to avoid abnormal changes in concentration of nutrients. The plants were harvested after 2 months' growth in the different solutions.

The effects of potassium deficiency on rice produced under the conditions of this experiment are shown in Tables XI and XII.

TABLE XI

*Effects of potassium deficiency on rice in solution culture*

(Average of 4 bottles)

Treatment	K p.p.m.	Height of plant cm.	Largest leaf		Tillers	Dry matter-grams		Length of roots cm.
			Length cm.	Maximum width mm.		Tops	Roots	
Full K	226	92	52	9	14	12.6	5.3	28
1/10 K	22.6	88	42	8	12	11.1	3.6	18
1/100 K	2.26	61	32	6	5	6.2	1.5	11
Minus K	..	45	24	5	3	2.6	0.6	8

TABLE XII

*Appearance of rice plants subjected to potassium deficiency in solution culture*

Treatment	Remarks on appearance of plants
Complete solution with full K	Plants were of a healthy green colour. Leaves showed no abnormality. The root system was pure white and had abundant secondary roots.
1/10 K	Plants were somewhat shorter than those grown with full K. The colour was normal green. Leaves were relatively shorter and showed a tendency to remain stiffly erect until fully expanded. There was some tip drying but no spotting or edge drying. Root system was reduced in size slightly and the secondary roots were limited in quantity.
1/100 K	Plants became greatly stunted with stems thicker than those grown with full K. Nodes were coloured brown when cut open. Leaves were dark green and stiff, showing a tendency to spread out at wide angles. There was no pigmentation on the leaf surface but the hook effect was present at the ends of some leaves which were almost erect. Tip drying was high. The older leaves died down rapidly but did not turn yellow before death. Root system was stunted and fragile with greyish colour. No secondary roots present.
Minus K	Plants turned yellow soon after transplanting but the colour rapidly changed to blue green. Plants remained highly stunted and squat shaped with short, stiff leaves which remained erect or horizontal. Hook effect was present (Plate II(a)). No pigmentation on leaves. Tip drying and edge drying high. Drying and rapid decay of older leaves with no yellowing. Rapid formation and decay of tillers. Nodes on stems were streaked brown (iron deposit) when cut open. Roots highly stunted and fragile with dark gray colour. No secondary roots.

It may be noted from Tables XI and XII that a moderate deficiency of potassium does not cause serious disturbances in the rice plant whereas an acute deficiency leads to the following effects: (1) stunting of the plant in stems, leaves and roots; (2) short, stout, soft stems; (3) reduction in

effective tillers though the process of tillering may go on readily; (4) stiffness of the leaves with a characteristic tendency to remain erect with the ends bent like hooks or to remain nearly horizontal; (5) change of colour to yellow in the early stage followed by change to the blue green colour which persisted throughout the growth period; (6) tip and edge drying of leaves without changing to yellow; (7) rapid decay of older leaves with no yellowing; and (8) tendency to undergo rotting, particularly noticeable in leaf sheaths and roots.

The results obtained in solution culture therefore generally confirm the observations made on plants grown in soil in pot culture.

### DISCUSSION

#### (i) *Morphological features*

As with other deficiencies a stunted condition is produced in rice grown under potassium deficiency. The height of the plant, leaf area and size of the root diminish progressively with the degree of deficiency as set out in Tables XI and XII. The internodal distance becomes shortened by potassium deficiency.

Rice shows characteristic leaf symptoms when subjected to potassium deficiency. These have been pointed out in connection with Table XII. Another feature noted in Table III shows that the leaves of rice suffer a progressive shortening in cases of potassium deficiency. This feature as well as the shortening of the internodes was pointed out by Kuilman (1936). A shortening of the internodes in sugarcane under potassium deficiency was observed by Hartt (1929).

Potassium deficiency causes the root system of rice to become stunted. Secondary roots fail to develop. The root as a whole tends to be brittle and discoloured, probably due to microbial attack.

#### (ii) *Colour symptoms*

Potassium deficiency has been found to produce two types of plants, namely, chlorotic, stunted plant with hard stems of normal shape on the one hand and dark green stunted plants with soft stems and squat shape on the other (Wall, 1939; Russell, 1942). The former type is produced when potassium deficiency is accompanied by a high concentration of sodium and a normal or low calcium content. The latter type of plant arises when there is little sodium but an excess of calcium. The conditions employed in the pot experiments with Patheingyi soil conform fairly closely to the second set named above and as might have been expected the rice plants were of the squat, sappy type with leaves of blue green colour. This colour

persisted practically unchanged till the plants were harvested. In older leaves patches of chocolate colour developed and appeared to be confined to the upper surface. The tips and edges of older leaves dried to a brownish red colour. The most noteworthy feature of the colour symptoms was that the leaves did not show any change into yellow colour either on drying or with advancing age. These symptoms are similar to those recognized on many plants, *e.g.*, Italian rye grass, potatoes, and tobacco (Eckstein, *et al.*, 1937). According to Schropp (1934) leaves of potassium-deficient rice exhibit a dark green colour. On the other hand Kuilman (1936) found that the older leaves of rice grown under potassium deficiency showed a characteristic yellow colour similar to that found in cases of nitrogen or phosphate deficiency. He obtained this effect in solution culture and in field trials. Contrary to the observation of Kuilman the author did not find any yellow colour in rice grown in the potassium-deficient Patheingyi soil or in the phosphate-deficient Kelington soil (Aiyar, 1946). In solution culture the yellow colour was obtained as a temporary phase which however soon changed to dark green. Apparently the development of yellow colour or dark green colour depends upon the nature of the nutrient medium. Wall (1940) identified an yellow type and a green type of tomato plant when grown in culture solutions. These types were found by Wall and Tiedjens (1940) to correspond respectively to nitrate—nitrogen or ammonium-nitrogen as the form of nitrogen present in the potassium deficient medium. Solutions containing both forms of nitrogen together might therefore give rise to a mixed type of plant which is first yellow and then green as observed by the author in solution culture (Table XII). According to Wall (1940) the chlorotic type is associated with the presence of an adequate supply of carbohydrates in the plant whereas the blue green type is conditioned by a low carbohydrate supply.

### (iii) *Yield of crop in relation to manuring*

The yield data of Table V show that untreated Patheingyi soil gives a poor yield of straw as well as grain and that when treated with potassium fertiliser high yields of straw and grain are produced.

It is widely believed that potash manuring does not increase the yield of rice. In fact there are reports that the use of potash even causes a reduction in yield. The large response to potash manuring shown by Patheingyi soil must therefore be regarded as an important finding as it allows the effect of potassium deficiency to be investigated in the soil. That this is a case of genuine potassium deficiency may be accepted from the following considerations, namely: (1) the direct response to potassium leading to high

yields whereas the response to phosphoric acid is negligible while the response to nitrogen occupies an intermediate position; (2) the presence of characteristic potassium deficiency symptoms in treatments N and NP which were prevented by potassium manuring; and (3) the significantly higher yield produced by NPK compared with that produced by N or NP. Owing to the high mobility of potassium within the plant, poor yield accompanied by abnormal composition and striking morphological symptoms need be expected only when there is acute deficiency of potash in the soil.

The beneficial effect of N and the relatively low effect of K when associated with P call for comment. In a case of potassium deficiency one would not expect N or P to produce any appreciable benefit. This expectation is borne out by the appearance of potassium deficiency symptoms in both these treatments.

Inspection of the yield data in Table V will show that the effect of K is much reduced when associated with P alone. When nitrogen is added to the combination PK the depressing effect of P is counteracted.

Although nitrogen treated rice plants grown in Patheingyi soil continued to show potassium deficiency symptoms till a late stage, the plants grew in size and matured normally producing relatively high yield of straw and grain. In experiments with potatoes on a potassium deficient soil Knowles, *et al.* (1940) obtained a significant response in yield to nitrogen treatment. The manner in which nitrogen acts will be considered later on.

In spite of the fact that nitrogen manuring may occasionally serve to produce an increased yield of rice in cases of potassium deficiency, it is necessary to point out that it is not desirable to use nitrogen in place of potash in such cases because (1) nitrogen is more expensive than potash on unit basis and (2) the quality of the crop is adversely affected by potassium deficiency. An important quality factor is the weight of individual grains which may be expressed for convenience in the form of 1,000-corn weight as is usual with barley. Some measurements obtained in connection with the pot culture experiment with Patheingyi soil are shown in Table XIII.

Apart from quality factors it is necessary to consider also the direct effect of potassium in determining the yield of grain. According to Ramiah (1937) rice yield is determined by the number of fruitful tillers, the number of grains per earhead, and the individual weight of the grain. Tillering is controlled by the nitrogen and phosphoric acid available to the plant provided other acute deficiencies do not exist. Moderate potassium deficiency has only a slight effect. Gregory (1937) has summarised the effects of these



three constituents on barley. When the deficiency of potassium is acute there is a large reduction in the fruitfulness of the tillers though the number of tillers may be quite high (Table II). This effect may be seen also by comparison of the straw-grain ratios of the yield data in Table V. The number of grains per earhead may be lowered by a shortening of the earhead which is usual in cases of nitrogen deficiency or phosphate deficiency. On the other hand, in cases of potassium deficiency the length of the earhead may be normal or short but the grains show a tendency to be widely spaced and chaffy in both types of earhead. This effect may be seen in Plate IV (b). The number of grains per earhead of rice found in the Patheingyi pot culture experiment are shown in Table XIII.

TABLE XIII

*Yield factors in rice grown in Patheingyi soil*

Treatment		Weight of 100 grains of unhulled rice (grams)	Number of grains per earhead
O	..	17.66	98
N	..	18.36	122
P	..	18.04	88
K	..	19.41	173
NK	..	19.31	166
PK	..	19.24	107
NP	..	18.24	124
NPK	..	19.09	160
Critical difference at 1% level		0.42	16.3

From a general consideration of the effects of nutrient elements on rice one may conclude that acute deficiency of potassium reduces the yield and quality of the crop seriously though a moderate deficiency may only produce slight deterioration in these respects and thus escape notice. The frequent reports that the use of potassium fertilizers gave disappointing results in increasing rice yields might be attributed partly to the lucky circumstance that acute potassium deficiency is probably not widespread though cases of moderate deficiency may be more general. It may also be remarked that the rate of application of the fertilizer is often too low to produce any result.

If a comparison is made (Table XIV) of the effects of certain major nutrients on rice it will be seen that potassium deficiency produces more serious deterioration than the others.

TABLE XIV

*Comparison of the effects of deficiency of four major nutrients on rice*

Deficiency		Colour	No. of tillers	Degree of fruitfulness of tillers	Maturity	Straw-grain ratio	Size of earhead	No. of grains in earhead
N	..	Chlorotic	Few	High	Hastened	Narrow	Very short	Few ; Close
P	..	Dark Green	Few	High	Delayed	Narrow	Short	Reduced ; Close
S	..	Chlorotic and dark green	Moderate	High	Delayed	Wide	Reduced	Reduced ; Close ; Chaffy
K	..	Chlorotic or dark green	High	Low	Delayed	Wide	Normal or short	Few ; Widely spaced ; Chaffy

(iv) *Composition of the crop*

The direct response to potassium treatment is reflected in the data shown in Table VIII according to which the potassium content of the crop, straw and grain, is greatly increased. The correlation between yield and potassium content of crop is highly significant (Table IX). In the absence of potassium there is a large increase in the percentage of nitrogen, phosphoric acid, calcium, magnesium and iron (Table VIII).

In so far as nitrogen accumulation is concerned potassium deficiency is similar to a deficiency of sulphur or phosphorus (Aiyar, 1945, 1946). In all these three cases, the high percentage of nitrogen must be the result of poor growth as well as ionic antagonism.

The accumulation of phosphoric acid in plants grown under potassium deficiency has been observed in tomatoes by Johnston and Hoagland (1929), Owen (1931), and Wall (1939). The same result has been observed in pineapple by Nightingale (1943) and in sugarcane by Hartt (1934). On the other hand, Thomas (1932), Jazvitzki (1945) and others speak of the reciprocal effects of N, P and K in plants. Rice is similar to these plants as potassium deficiency leads to accumulation of phosphoric acid (Table VIII) though it is interesting to note that phosphoric acid deficiency causes a depression in the absorption of potash (Aiyar, 1946).

There is a large volume of literature on the antagonism between potassium on the one hand and calcium and magnesium on the other. Ehrenterg (1919) pointed out the existence of an inverse relation between potassium and calcium in plants, Ishizuka (1935) verified this relation in the case of rice in solution culture. Thomas and Mack (1939) found that the absorp-

tion of calcium, magnesium and potassium by plants followed a linear law. An inverse relation between potassium and magnesium may thus be assumed to exist. Drake and Scarseth (1939) found this to be true for tobacco while Walsh and Clarke (1945) found that potassium depresses the absorption of magnesium in tomato. From the data of Table VIII it would appear that the inverse relation between potassium and magnesium is striking but the effect on calcium is not so characteristic. Whether the depressing action of potassium affects both calcium and magnesium equally or unequally seems to depend on the nature of the soil. In Patheingyi soil the exchangeable magnesium is abnormally high compared to calcium (Table I).

Hoffer (1926, 1930) observed an accumulation of iron in the nodes of potassium-deficient maize plants and used this effect as a test for potassium deficiency. According to Willis and Pland (1934), however, this test is not specific. The existence of a strong antagonism between potassium and iron has been confirmed by Eckstein and Jacob (1929), Shibuya and Torii (1935), Scharrer and Schropp (1936), Rohde (1940) and others. In rice plants grown in unmanured Patheingyi soil the nodes were found to be rust coloured when cut open and readily responded to the Hoffer test for iron. The plants manured with potash did not show any accumulation of iron.

#### (v) *Metabolic changes*

In cases of potassium deficiency it is well established that the metabolism of nitrogen and carbohydrates is abnormal. Burrell (1926) found an increase of soluble nitrogen in potassium deficient soybean plants. Nightingale, *et al.* (1930) found a large accumulation of soluble nitrogen in tomato grown in potassium-deficient solution. In potassium deficient tobacco plants Day (1940) found lower percentages of starch than in the fully manured plants. In pineapple plants grown under potassium deficiency Nightingale (1942) found that the carbohydrate contents were low and pointed out that the fruitfulness was not controlled by the absolute percentage of starch in the plant but by the nitrogen-starch balance. According to him the deeper the green colour of the plant the greater is the deficiency of starch though the absolute amount of starch may be high. Potassium manuring or a high potassium status in the soil is considered to be essential for the formation of plump, well filled grain in cereals. This plumpness apparently depends on the deposition of high amounts of starch in the grain. In rice plants grown in Patheingyi soil, the nitrogen and carbohydrate fractions were determined before flowering and the starch contents in the mature grain. These data are shown in Tables XV and XVI respectively.

TABLE XV  
*Nitrogen and carbohydrates in rice plants grown in Patheingyi soil*  
(Percentages on oven dry basis)

Samples of plant collected on 15-10-1941	Nitrogen fractions			Carbohydrate fractions	
	Soluble N	Protein N	Total N	Soluble sugars as glucose	Starch
Unmanured ..	0.35	1.50	1.85	2.07	2.89
Manured with K <sub>2</sub> SO <sub>4</sub> ..	0.12	0.66	0.78	7.54	10.13

TABLE XVI  
*Starch contents of unhulled rice grain grown in Patheingyi soil*  
(Percentages on oven dry basis)

Treatment	Starch	Potash	Nitrogen
O ..	52.7	0.25	1.771
N ..	59.1	0.32	0.959
P ..	55.8	0.21	1.463
K ..	62.6	0.36	0.945
NK ..	62.1	0.37	0.931
PK ..	61.0	0.32	1.015
NP ..	58.1	0.31	1.001
NPK ..	60.9	0.38	1.043

From the data of Table XV it will be seen that there is a large accumulation of soluble —N, protein —N and total —N and a deficiency of carbohydrates in the unmanured rice plants and that manuring with potassium sulphate has lowered all forms of nitrogen and increased the carbohydrates in the plant. On the whole, the effect of potassium deficiency on metabolism in rice are very similar to those found in other plants.

A close parallelism is seen to exist between the starch content and percentage of potassium in rice grain (Table XVI). In researches on the malting quality of barley Russell and Bishop (1933) showed that the nitrogen-free extract of malt was inversely correlated with the nitrogen content of barley. A similar relation exists in rice grain though there are a few exceptions when nitrogen manures were used without added potash. This observation suggests that direct manuring with potash is superior to the indirect action of nitrogen in maintaining the quality of the rice crop grown in Patheingyi soil.

There is another aspect of potassium manuring which deserves to be noted. Although a high potassium content of the crop may lead to high yields in cases of deficiency and to high quality in the grain crop the nutritive value of straw is lowered by high potassium (Bear, *et al.*, 1945). It is, therefore, desirable to restrict the supply of potassium to the lowest possible level consistent with yield not only as a measure of economy but as a precaution to preserve the nutritive value of the crop.

(vi) *Soil conditions and potassium deficiency*

It is generally accepted that available potassium (Dyer) or exchangeable potassium may be taken as an index of the potassium supplying power of the soil. There are however, many instances in which crop response does not agree with the chemically determined potassium status of the soil. According to Nightingale (1943) "there is no single common factor that can be employed as a guide for potash applications, unless it is the plant itself, which supplies an integration of all environmental influences."

Judged by chemical analysis (Table I) Patheingyi soil is quite rich in available or exchangeable potassium. Powerful factors must, therefore, be at work to produce potassium deficiency in crops grown in this soil. Three groups of factors may be involved, namely, (i) fixation in inorganic forms, (2) assimilation by micro-organisms, and (3) ionic antagonism.

Potassium fixation has been shown to be due to (a) conversion into muscovite (Volk, 1934); (b) non-reversible entry into the crystal lattices of montmorillonite (Joffe and Kolodny, 1937), bentonite and other minerals (Truog and Jones, 1938); (c) precipitation as double phosphates of iron or aluminium (Joffe and Kolodny, 1936). These processes however proceed slowly and cannot account for the loss of substantial quantities of available potassium.

Assimilation of potassium by micro-organisms has been pointed out as a possible means of causing potassium deficiency (Jenny and Shade, 1934; Lamb, 1935; Dean, 1935, 1936; Blume and Purvis, 1939). This process is of a dynamical character as it is effective in the growing season and is reversed when the soil dries out. The conditions for the prolific development of microflora (bacteria, actinomyces, protozoa, saprophytic and blue green algæ) are present in the Patheingyi soil, namely (i) available energy material as indicated by the wide carbon-nitrogen ratio (18:1) and amounts of crop residues unavoidably present; (ii) favourable soil reaction and calcium status (Table I); and (iii) high nutrient status. Potassium present in living mycelium or tissue is insoluble but when the tissues dry out or

undergo decomposition it becomes soluble once again. It appears to be highly probable that microbial absorption in competition with the growing plants is the main cause of the potassium deficiency in Patheingyi soil. The beneficial action of ammonium sulphate may be explained partly on its effect in facilitating the biological decomposition of microbial mycelium thereby releasing potassium in soluble form.

Ionic antagonism is another possible cause leading to potassium deficiency in Patheingyi soil. It is well established that relatively high proportions of calcium, magnesium, ammonium and iron tend to repress potassium absorption by plants. Thus potassium deficiency is often encountered in calcareous and other calcium-rich soils (Sears, 1930; McRae, 1932; Lamb, 1935; Allaway and Pierre, 1939). The antagonism between calcium and magnesium on the one hand and potassium on the other, has been referred to already as a possible factor in causing potassium deficiency. Again antagonism between ammonium ions and potassium is fairly universal in plants but rice appears to be an exception in this respect (Pirschle, 1931). On the other hand, the favourable effect of nitrogen in increasing the yield of rice and in assisting the crop to absorb more potash from Patheingyi soil is clearly brought out in Tables V and VIII. Rippel (1927) observed this phenomenon in connection with researches on Mitscherlich growth curves. One suggestion to explain the beneficial action of ammonium sulphate is that it hastens biological decomposition of mycelium. The second suggestion is based on the ionic antagonism between the positive ammonium ions on the one hand and calcium and magnesium on the other (Willis and Piland, 1931; Lewis, 1933; Burstrom, 1934). Although the relatively low percentages of calcium and magnesium and the high percentage of potassium in straw manured with ammonium sulphate (Table VIII) indicate that the antagonism of ammonium is apparently effective in keeping down the entry of calcium and magnesium and thereby favouring the absorption of potassium by the rice plant greater emphasis must be placed on the biological effect.

One may thus conclude that several factors are favourable in Patheingyi soil for causing a deficiency of potassium. The addition of potassium sulphate as fertilizer overcomes these negative factors and supplies the plant with readily available potassium, even though the quantity added represents only .06 milliequivalents of potassium per 100 grams of soil. Ammonium sulphate added as fertilizer also helps the plant to overcome these adverse conditions and acquire sufficient potassium to produce a relatively high yield. The effect of nitrogen is however much slower than that produced by the direct use of potassium sulphate because some period must elapse

before the microbial organic matter is decomposed and sufficient potassium released for the use of the growing plant. This effect of nitrogen is of great agronomic significance and might account partly at least for the widely observed failure of potash to produce yield increases in rice when it is used in conjunction with ammonium sulphate as a fertilizer. Finally it may be added that the discussions and conclusions arrived at in this article have reference only to Patheingyi soil and may not be applicable to all soils without appropriate modifications.

### SUMMARY

1. The causes of certain abnormalities and poor diseased condition of rice grown in Patheingyi township, near Mandalay, Burma were investigated by pot culture and solution culture experiments. The condition was shown to be caused by potassium deficiency.

2. The following characteristics were shown by rice plants grown in unmanured soil in pots; (a) stunted bushy plants with blue green colour; (b) short sappy stems; (c) short stiff leaves which were erect ending in a hook-shaped curvature or which were spread out practically at right angles to the stem ending in a wide upward curvature; (d) chocolate coloured patches on leaves; (e) progressive shortening of the internodes and leaves from the oldest to the youngest; (f) high proportion of leaf drying and decay of the older leaves and leaf sheaths; (g) complete absence of yellow colour during drying; (h) continued tillering throughout the life of the plant with a high percentage of decay; (i) serious reduction of grain formation; and (j) wide spacing of grains on the panicle. Experiments in potassium-deficient culture solutions confirmed most of the morphological and colour symptoms.

3. Manurial experiments showed that application of potassium sulphate prevented the appearance of any of the deficiency symptoms observed in plants grown in unmanured soil. The rice plants manured with potassium sulphate were tall, healthy and normal in all respects. Large increases in yield of straw and grain were obtained by manuring with potassium sulphate alone.

4. Manuring with ammonium sulphate produced large increases in growth but the deficiency symptoms persisted practically until the plants matured. Grain and straw yields were only somewhat less than those produced by potash manuring. This indirect effect of nitrogen in the form of ammonium sulphate appeared partly to be due to its antagonism to the absorption of calcium and magnesium but mainly due to its action in helping

to break down organic matter in which potassium had been locked up in unavailable form.

5. Phosphoric acid was found to depress the action of potash when these two nutrients were used together.

6. In the absence of added potash grain formation was very seriously reduced and the straw-grain ratio was abnormally high. When normal growth and development took place by the use of potash or nitrogen this ratio varied from 0.90 to 1.40 as against 2.25 to 4.57 when potassium deficiency was present.

7. The composition of the crop showed that in the absence of potassium the percentage of potash was abnormally low whereas the percentages of nitrogen, phosphoric acid, calcium, magnesium and iron were abnormally high. Manuring with potassium sulphate caused a large increase in the potash content of the crop accompanied by a corresponding lowering of nitrogen, phosphoric acid, calcium, magnesium and iron; the effect of nitrogen was similar to that of potash though it was more slowly exerted.

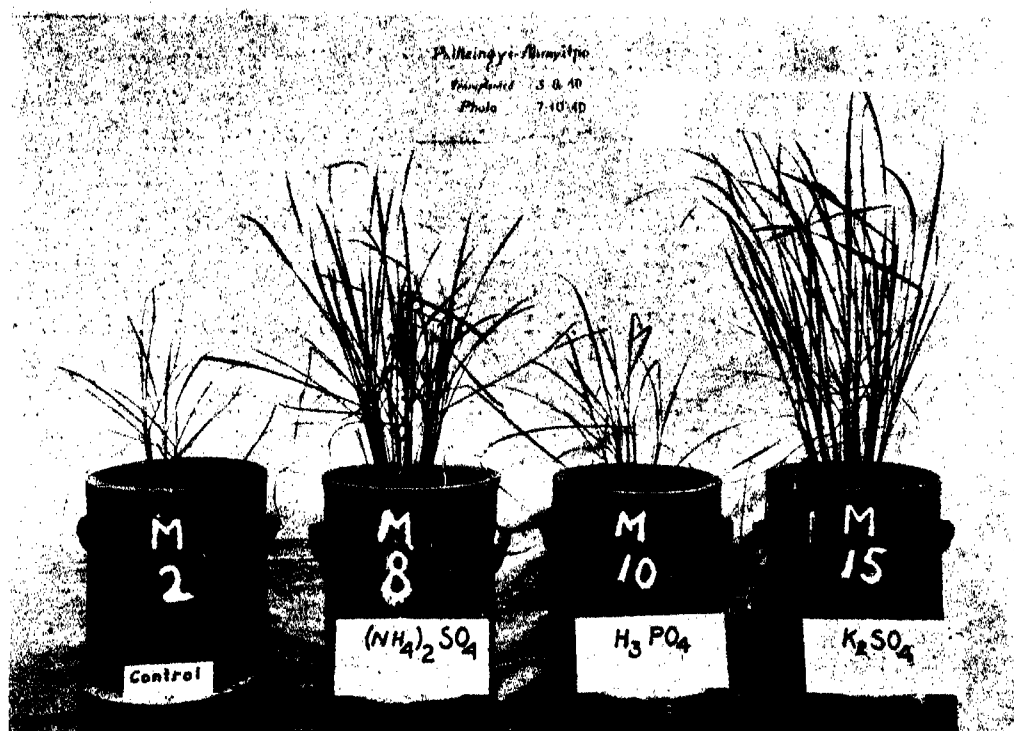
8. The causes for the development of potassium deficiency in Patheingyi soil are discussed. It is suggested that the depression of potassium absorption by plants induced by the ionic antagonism of calcium and magnesium on the one hand and assimilation of an abnormally high proportion of potassium by the large increase in soil microflora on the other are the main factors involved in this unexpected phenomenon in this soil which is apparently rich in potassium as judged by chemical methods.

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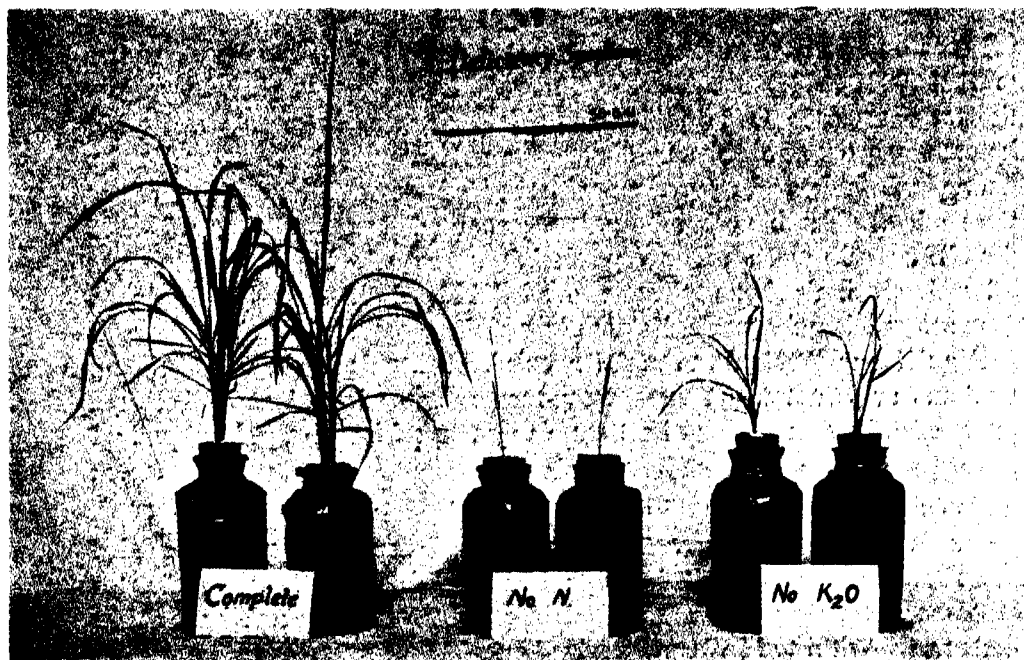
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a and b—Response of rice to nutrients added to Patheingyi soil.



*a.* Effect of Potassium deficiency on rice in culture solution.



*b.* Effect of Potassium deficiency on earheads of rice grown in Patheingyi soil.

# DISEASES OF PAN (*PIPER BETLE*) IN SYLHET, ASSAM\*

## Part VII. Effects of Some Soil Treatments on the Incidence of Sclerotial Wilt

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### I. INTRODUCTION

*Sclerotial wilt of pan* (*Piper betle*, L.) due to *Sclerotium rolfsii* Sacc. is a serious disease of this crop in certain *pan*-growing tracts of Assam (Chowdhury, 1945). Soil treatments have proved successful in the control of several plant diseases, especially those which are due to soil-inhabiting parasites. The most familiar example is that of the control of clubroot disease of crucifers by making the soil alkaline by the addition of lime (Chupp, 1928; Wellman, 1930). Doran (1927, 1928, 1929 and 1931) and Morgan and Anderson (1927) reported the reduction of *Theilaviopsis* black root-rot of tobacco and *Phymatotrichum* cotton root-rot by increasing soil acidity. The severity of root rot of wheat seems to be influenced by the time and mode of tillage (Sewell and Melchers, 1924) and the use of fertilizers has been known to markedly control *Aphanomyces* root rot of peas (Walker and Musbach, 1939). In New Jersey root rot of peas are reduced by heavy applications of hydrated lime (4000 lbs. per acre) but lesser amounts had no inhibitory effect (Hänseler, 1927) and elsewhere incidence of the rot was delayed and injurious effects on the host greatly reduced by use of 1,000-2,000 lbs. of complete fertilizers per acre (Hänseler, 1931). Reduction in root rot of sugar beets has been reported with applications of commercial fertilizer containing 100 lbs. nitrogen per acre (Leach, 1941) and promising results in control of black-root of sugar beet were secured by incorporation with the seed of liberal amounts of phosphate fertilizer, three to four times the customary dose of 100 or 150 lbs. per acre (Coons, *et al.*, 1941).

Control of potato scab by green manuring with grass cuttings was first reported by Millard (1921). Field control of the take-all disease of wheat in Kansas by the application of such organic materials as chicken and horse

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\* This work was carried out at the Plant Pathological Laboratory, Sylhet.

manure, green alfalfa, boiled oats and barley kernels, and potato flour was very briefly reported by Fellows (1929). Garrett (1936, 1937, 1938 and 1940) has attempted to analyse this controlling effect of organic materials upon the take-all disease and has suggested that addition of organic material to the soil would depress the parasitic activity of *Ophiobolus graminis* in so far as it increased the concentration of carbon dioxide in the micro-atmosphere around the roots. Working on the same disease, Stumbo, *et al.* (1942) and Clark (1942) have recently emphasised the beneficial manurial effect of such organic supplements in increasing the resistance to attack of the wheat plant. In Arizona King, *et al.* (1934) and King (1937) reported control of the cotton root-rot fungus *Phymatotrichum omnivorum* by burying large quantities of organic matter in deep furrows in the affected areas.

Petri (1929) recommended drainage and manurial dressings counter-balanced by applications of phosphate for prevention of *Phytophthora* root-rot of citrus. Fedorintchik (1935) reported that four-year rotations, in which cruciferous crops were kept off the land for three years, were sometimes adequate for the control of *Plasmidiophora brassicae*, but a five-year rotation was usually found to be the minimum that can safely be employed. Ratcliffe (1934), Rogers (1937) and Rea (1939) reported that a four-year rotation markedly reduced losses in yield of cotton due to the attack of the cotton root rot fungus *Phymatotrichum omnivorum*. Goss and Afanasiev (1938) reported satisfactory control of the potato scab fungus, *Actinomyces scabies* by four- to six-year rotations which eliminating the severe form of scab, gave the highest proportion of sound, unmarked tubers. Leach (1941) reported that a two- to four-year rotation of sugar beets with cereals, or winter crops of peas, spinach or lettuce reduced *Sclerotium rolfsii* to save levels. Maxson (1939) reported that root rots of sugar beets caused by *Rhizoctonia solani* was not present in beet fields preceded by four years of gram.

During the past few years experiments were carried out to determine the effects of certain soil treatments on the incidence of this disease. As the parasite is a soil-dweller it was supposed that certain soil treatments might be found effective in partially or completely controlling the disease. With this idea in view certain experiments were conducted during the years 1940-46 and the results so far obtained are reported in this paper.

## II. EXPERIMENTAL

All the experiments reported below were conducted at Sylhet, Basudevsvri and Kumrakapan on land which was heavily and uniformly infected with

the disease in order to get reliable and comparable data. Randomised block system of layout was always followed and the treatments were duly replicated.

(i) *Effects of Ploughing*.—An experiment to study the effect of depth of ploughing on the incidence of *sclerotial* wilt of *pan* was carried out. Five isolated fields in which *pan* plants had died of *sclerotial* wilt were selected. Each field was divided into four plots, and each plot was 40 × 12 feet. Four treatments, i.e., three inch deep ploughing, six-inch deep ploughing, nine-inch deep ploughing and twelve-inch deep ploughing were replicated five times. Thereafter equal number of healthy *pan* setts was planted in each of these plots in May, 1942 and the plants were given the same care and treatments with respect to manuring, earthing and other cultural operations. The number of deaths occurring in each of the plots was carefully and regularly noted during the years 1942–43 and 1943–44. In every case the dead plants were carefully examined to see that the death was due to the attack of *S. rolfsii*. The results obtained are recorded in Table I.

TABLE I  
*Effect of depth of Ploughing on the Sclerotial wilt of Pan*

Treatments	Total number of setts planted	Average percentage of death		
		1942–43	1943–44	1944–55
3 inch deep ploughing	1250	12.8	14.1	16.7
6-inch do	1250	7.8	7.5	8.2
9-inch do	1250	5.3	5.0	4.8
12-inch do	1250	2.4	3.6	2.8

It will be evident from the data presented in Table I that deep ploughing considerably decreased the percentage of death of the *pan* plants due to *S. rolfsii*. In a previous paper the author (Chowdhury, 1945) has communicated that the parasite loses its infective power when its sclerotia are buried three inches or more in the soil. Deep ploughing was therefore meant to bury the sclerotia of the parasite to depths where they would lose their infective power and the results presented in Table I show that the more the depth of ploughing the more effective was the burial and consequently less the percentage of death. But it will, however, be seen that even by ploughing the fields to a depth of 12 inches it was not possible to completely control the disease. It may be remarked in this connection that by deep

ploughing it is not always possible to bury all the sclerotia beyond a depth of three inches. Ploughing, cross-ploughing, laddering and heavy monsoon rains, which wash much of the surface soil might bring some of the sclerotia at the surface or an inch or two below the surface. But in spite of this limitation it will be seen from the results presented in Table I that deep ploughing is sufficiently effective in controlling the disease. The efficiency of this method can be greatly increased by frequently piling up disease-free earth along the ridges, thus helping the burial of the sclerotia which might come to the surface beyond a depth of three inches. Piling up of earth along the ridges on which the *pan* plant is allowed to grow is a regular cultural practice with the *pan* growers (Chowdhury, 1944).

(ii) *Effect of growing other Crops before planting Pan.*—An experiment was carried out to see to what extent it was possible to suppress the parasite in the infected fields by growing different crops and thereby to control the disease. Four fields uniformly infected by the parasite were selected for the purpose. Each field was then divided into five plots and each plot was  $45 \times 15$  feet. Five treatments were tried and each treatment was replicated four times. The treatments were as follows:

(a) *Ulu grass (Imperata arundinacea)* was planted in April, 1940 and allowed to grow in the plots during the years 1941, 1942 and 1943. In January, 1944 the grass was cut and the plots thoroughly ploughed and prepared for the planting of *pan*.

(b) In March, 1940 *aus paady (Dumai)* was sown and harvested in June, 1940; thereafter *sail paady (Latisail)* was transplanted in July, 1940 and harvested in December, 1940. The same cropping programme was followed during the years 1941, 1942 and 1943. In January, 1944 the plots were carefully ploughed and prepared for the planting of *pan*.

(c) Potato was planted in October, 1940 and harvested in January, 1941. In March, 1941 jute was sown in these plots and harvested in August 1941. In October, 1941 chillies were planted and harvested in May, 1942. In June, 1942 *til (Sesamum orientale)* was planted and harvested in October, 1942. In November, 1942 potato was planted and harvested in February, 1943. Jute was then sown in March, 1943 and harvested in August, 1943. Potato was then planted in October, 1943 and harvested in January, 1944. Thereafter the plots were thoroughly ploughed and prepared for the planting of *pan*.

(d) Tobacco was planted in October, 1940 and harvested in March 1941. In April, 1941 *mukhi (Colocasia antiquorum)* was planted and harvested in August, 1941. Tobacco was then planted in October, 1941 harvested and followed by *mukhi*. The same cropping scheme was followed

during the years 1942 and 1943. In 1944 after the harvest of tobacco in February, the plots were thoroughly ploughed and prepared for the planting of *pan*.

(e) *Pan* was planted in July, 1940 and allowed to grow during the years 1941, 1942 and 1943. In January, 1944 the plots were cleared of the *pan* crop, thoroughly ploughed and prepared for the planting of *pan*.

The different crops grown during the years 1940–44 were kept under careful observation and the number of deaths as it occurred due to the attack of *S. rolfsii* was noted. The percentage of death noted in the different crops is recorded in Table II.

TABLE II  
Percentage of death in the different crops during 1940–44

Treatment	Crops	Percentage of death				
		1940	1941	1942	1943	1944
(a)	<i>Ulu grass</i>	Nil	Nil	Nil	Nil	Nil
(b)	<i>Dumai paddy</i>	2.0	0.75	Nil	Nil	Nil
	<i>Sail paddy</i>	1.5	0.80	Nil	Nil	Nil
(c)	Potato	4.5	..	..	2.8	3.0
	Jute	..	Nil	..	Nil	..
	Chillies	..	..	3.4	..	..
	<i>Til</i>	..	..	4.0	..	..
(d)	Tobacco	Nil	Nil	Nil	Nil	Nil
	<i>Mukhi</i>	..	..	..	..	..
(e)	<i>Pan</i>	14.6	16.0	15.8	19.2	..

It will be evident from the data presented in Table II that *ulu grass*, jute, tobacco and *mukhi* remained free from the attack of *S. rolfsii* all throughout; paddy was very slightly attacked only during the first two years, from the third year it was also free from the invasion of the parasite. Potato, *til* and chillies were attacked by the parasite but only very slightly. Of all crops grown *pan* was the most severely attacked and the extent of mortality was practically the same in all the years except 1943 when it reached 19.2 per cent.

In May, 1944 all these plots were carefully ploughed and the soil prepared for the planting of *pan*. Planting was done in July, 1944. Only healthy *pan* setts were planted. Equal number of setts were planted in each of the plots and the planting was done at the same time and on the same day. Plants were thereafter given the same care and attention in respect to manuring, irrigation and other cultural operations.



The *pan* plants were kept under careful observation. The number of deaths was regularly noted and all the dead plants were carefully examined to definitely ascertain that the death has been due to the invasion of the plants by *S. rolfsii*. The average percentage of death observed during the years 1944-45, 1945-46 and 1946-47 is recorded in Table III.

TABLE III  
Average Percentage death of *pan* plants

Treatments	Crops grown during the years 1940-44	Total number of <i>pan</i> setts planted	Average percentage of death		
			1944-45	1945-46	1946-47
(a)	<i>Ulu</i> grass ..	1400	Nil	Nil	Nil
(b)	<i>Aus</i> and <i>sail</i> paddy ..	1400	Nil	Nil	Nil
(c)	Potato, Jute, Chillies and <i>til</i> ..	1400	4.6	5.4	7.2
(d)	Tobacco and <i>mukhi</i> ..	1400	Nil	Nil	Nil
(e)	<i>Fan</i> ..	1400	17.2	16.6	18.5

It will be evident from the data presented in Table III that continuous cropping for four years with *ulu* grass, paddy and tobacco and *mukhi* completely controlled the parasite in the soil and gave a healthy crop of *pan* when planted afterwards. The other crop grown failed to completely control the disease but decreased it to save levels and gave a percentage of death far less than what was observed in the plots where *pan* was continuously grown for years.

(iii) *Effect of Organic Manures*.—The effect of a few organic manures on the incidence of the disease was studied. Five old *pan boroj* fields uniformly infected with the parasite was selected for the purpose. Each of these fields was then divided into four equal plots, each plot being 45 × 15 feet. Four treatments were tried and each treatment had five replications. The treatments were as follows:

- (a) Rotten cowdung applied at the rate of 15 tons per acre.
- (b) Rotten cowdung applied at the rate of 25 tons per acre.
- (c) Water hyacinth and cowdung compost applied at the rate of 25 tons per acre.
- (d) No manure applied.

Equal number of healthy *pan* setts was planted in each of the plots in July, 1942. The plants were therefore given the same treatments with respect to manuring, earthing and other cultural operations. The number of deaths

occurring was regularly noted and all the dead plants were minutely examined to see that the deaths were due to the attack of *S. rolfsii*. The average percentage of deaths noted is recorded in Table IV.

TABLE IV  
*Effect of organic manures on the incidence of the disease*

Treatments	Total number of setts planted	Average percentage of death		
		1942-43	1943-44	1944-45
(a) Cowdung at the rate of 15 tons per acre	1500	9.8	10.2	12.5
(b) Cowdung at the rate of 25 tons per acre	1500	6.5	8.9	7.2
(c) Water hyacinth and cowdung compost at the rate of 25 tons per acre	1500	6.9	8.4	8.1
(d) No manure : control .. ....	1500	15.6	17.2	16.9

It will be evident from the data presented in Table IV that additions of large doses of cowdung and composts reduced the percentage of disease incidence. The percentage of death in the plots which received cowdung and composts was far less than in the control plots; further the percentage of death in the plots which received 25 tons of manure per acre was less than that in the plots which received 15 tons per acre. Similar results have been obtained by other workers as well. Thus Sanford (1926) reports control of *Actinomyces scabies*, Millard and Taylor (1927) control of *Fusarium lini*, McRae and Shaw (1933) control of *F. vasinfectum*, Fellows (1929) control of *Ophiobolus graminis* and King, *et al.* (1934) control of *Phymatotrichum omnivorum* by the application of organic matter in the soil.

(iv) *Effect of Green Manures.*—The effect of green manures on the incidence of sclerotial wilt of pan was also studied. Five old pan boroj fields uniformly infested with the parasite, were selected. Each of these fields was then divided into four equal plots, each plot being 45 × 15 feet. Four treatments were tried and each treatment had five replications. The treatments were as follows:

(a) *Sunn hemp (Crotalaria juncea)* was grown for two years, 1940 and 1941. The crop was grown as usual during the rains and ploughed under. Sowing was done in May and ploughing under in July. During the rest of the year the plots remained fallow.

(b) *Dhaincha (Sesbania cannabina)* was grown for two years, 1940 and 1941. The crop was grown as usual during the rains and ploughed under,

Sowing was done in May and ploughing under in July. During the rest of the year the plots remained without any crops.

(c) *Cowpea* (*Vigna catjang*) was grown for two years, 1940 and 1941. Sowing was done in May and the crop was ploughed under in July. During the rest of year the plots remained fallow.

(a) *Pan* was grown during the years 1940 and 1941. The crop was not ploughed under.

The green manuring crops grown were under careful observation. None of these crops was attacked by *S. rolf sii*. The *pan* crop, however, showed deaths due to the attack of the parasite; the average percentages of mortality were 14.8 and 17.2 for the years 1940 and 1941 respectively.

In April, 1942 the *pan borojes* in the different plots were destroyed and these plots along with the other plots where green manuring was done were ploughed and prepared for the planting of *pan*. In July, 1942 equal number of healthy *pan* setts were planted in these plots. The plants were thereafter given the same treatments with respect to manuring, earthing and other cultural operations. The number of deaths occurring was regularly noted and the dead plants were minutely examined to see that the deaths were due to the attack of *S. rolf sii*. The percentage of death noted is recorded in Table V.

TABLE V  
*Effect of Green manuring on the incidence of S. rolf sii*

Treat- ments	Green manuring crops grown	Number of pan setts planted	Average Percentage of death		
			1942-43	1943-44	1944-45
(a)	<i>Sunn hemp</i>	1500	4.2	4.7	5.2
(b)	<i>Dhaincha</i>	1500	3.7	3.4	4.5
(c)	<i>Cowpea</i>	1500	4.4	4.9	5.6
(d)	<i>Pan</i> : control	1500	15.2	14.9	16.5

It will be evident from the data presented in Table V that the percentage of death was comparatively less in the plots in which green manuring was done than in those where green manuring was not done but, on the other hand, *pan* was allowed to grow.

(v) *Effect of Minor Elements*.—An experiment was conducted to see the effect of certain minor elements on the incidence of the disease. Five

fields which were almost uniformly infested with the parasite were selected. Each of the fields was divided into six equal plots, each plot being 40 × 15 feet. The plots were then given the following treatments and each treatment had five replications. The treatments were as follows:—

- (a) Zinc sulphate was applied at the rate of 10 lbs. per acre.
- (b) Copper sulphate was applied at the rate of 10 lbs. per acre.
- (c) Ferrous sulphate was applied at the rate of 10 lbs. per acre.
- (d) Manganous sulphate was applied at the rate of 10 lbs. per acre.
- (e) Magnesium sulphate was applied at the rate of 10 lbs. per acre.
- (f) Control.

All these salts were ground fine and mixed with the soil. Equal number of healthy pan setts were thereafter planted in each of the plots. All the plants were given the same treatments subsequently in regard to manuring, earthing and other cultural operations. The number of deaths occurring was regularly noted and the dead plants examined to see that the death was due to *S. rolfsii*. The percentage of death observed is recorded in Table VI.

TABLE VI  
*Effect of Minor elements on the incidence of the disease*

Treatments	Number of Pan setts planted	Average percentage of death		
		1942	1943	1944
Zinc sulphate ..	1200	14.4	15.0	12.9
Copper sulphate ..	1200	13.2	15.1	13.7
Ferrous sulphate ..	1200	14.9	16.2	15.2
Manganous sulphate ..	1200	14.0	16.7	14.9
Magnesium sulphate ..	1200	16.2	14.4	15.4
Control ..	1200	15.0	15.9	16.2

It will be evident from the data presented in Table VI that none of the minor elements applied had any influence on the incidence of the disease. The percentage of death of *pan* plants was practically the same in the control plots and in the plots which received an application of the salts of the minor elements.

(vi) *Effect of Fertilizers*.—In a previous paper the author (Chowdhury, 1946) has reported the effect of a few manures on the sclerotial wilt of *pan*. In this paper the results of further studies made by him are reported. The effect of a number of fertilizers on disease incidence was tested in uniformly

infected fields. Each treatment had five replications. Plots were of equal size and only healthy *pan* setts were planted in July, 1942. Plants were kept under observation and deaths were recorded regularly. The data of the experiment are summarised in Table VII.

TABLE VII  
*Effect of Fertilizers on the incidence of the disease*

Treatments	Rate per acre	Number of plants	Average percentage of death	
			1942-43	1943-44
1 Untreated control	..	1200	16.2	17.6
2 Ammonium sulphate and superphosphate	200	1200		
	150		7.2	9.6
3 Ammonium sulphate, superphosphate and potassium chloride	200	1200		
	150		5.6	0.2
	75			
4 Ammonium sulphate	200	1200	8.4	7.6
5 Superphosphate	150	1200	12.9	14.2
6 Potassium chloride	75	1200	11.2	14.8
7 Superphosphate and potassium chloride	150			
	75	1200	10.7	13.9
8 Ammonium sulphate and Potassium chloride	200			
	75	1200	8.9	6.2

It will be evident from the data presented in Table VII that the percentage of death of the *pan* plants was always less in the plots which received fertilizers than which had not. Ammonium sulphate singly or in combination with superphosphate and potassium chloride or either of the latter was found almost equally effective in reducing the percentage of mortality. Superphosphate and potassium chloride singly or in combination were found less effective in reducing the percentage of death than ammonium sulphate singly or in combination with superphosphate or potassium chloride or both.

(vii) *Effect of Soil Amendments.*—The effect of varying doses of lime and gypsum on the incidence of the disease was studied. The experiment was conducted on uniformly infected fields and each of the treatments had five replications. Plots were of equal dimensions and equal number of only healthy setts were planted in each of the plots. The number of deaths was regularly noted and the data of the experiment are summarised in Table VIII.

It will be evident from the data presented in Table VIII that the different soil amendments tried exerted practically no effect on the incidence of the disease. The percentage of death in the control and in the treated plots was practically the same from practical point of view.

TABLE VIII

*Effect of Soil Amendments on the incidence of the disease*

Treatments	Rate per acre	Number of plants	Average percentage of death	
			1942-43	1943-44
	lbs.			
1 Calcium carbonate	410	1200	13.0	14.9
2 Calcium oxide	410	1200	12.5	14.4
3 Calcium chloride	410	1200	16.3	14.9
4 Calcium sulphate	410	1200	14.6	15.2
5 Calcium carbonate	820	1200	16.6	15.3
6 Calcium oxide	820	1200	14.2	14.6
7 Calcium chloride	820	1200	14.6	15.2
8 Calcium sulphate	820	1200	15.9	16.0
9 Control	..	1200	16.2	15.0

## III. SUMMARY AND CONCLUSIONS

The results of the experiments carried out during the years 1940-46 to ascertain the effects of some soil treatments on the incidence of the *Sclerotial* wilt of *pan* due to *S. rolfsii* are presented.

It has been found possible to considerably reduce the percentage of mortality by deep ploughing and by the growing of other crops for a few years on the infested soil. Continuous cropping for four years with *ulu* grass, paddy or tobacco and *mukhi* completely controlled the disease.

Applications of organic manures, cowdung and composts appreciably reduced the percentage of death due to the parasite.

Turning under of green manures has also been found beneficial in suppressing the disease.

Applications of zinc sulphate, copper sulphate, ferrous sulphate, manganous sulphate and magnesium sulphate have been found ineffective in minimising or controlling the disease.

Of the fertilizers tried, ammonium sulphate alone or in combination with superphosphate or potassium chloride or both the latter was found effective in appreciably suppressing the disease. Superphosphate or potassium chloride when applied singly or in combination exerted less effect but kept the mortality always below that observed in the control plots.

None of the soil amendments tried exerted any influence.

It may be concluded from these studies that the *Sclerotial* wilt of *pan* as it occurs in Assam can be effectively controlled by deep ploughing, by

green manuring, by the application of organic manures and fertilizers and by the growing of other crops in the infested fields for a few years.

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# DISEASES OF PAN (*PIPER BETLE*) IN SYLHET, ASSAM\*

## Part VIII. Effect of Temperature on the Development of *Sclerotial Wilt of Pan*

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### I. INTRODUCTION

SOIL temperature has been known to exercise an important effect on the occurrence of soil-borne diseases. Studies so far made by a number of workers have revealed that each of those diseases has an optimum soil temperature for its maximum development and it causes the greatest damage only at such temperatures. Thus Tisdale (1923) found that the optimum temperature for the development of cabbage yellows was approximately 26° C. whilst the optimum temperature for the growth of the causal fungus *Fusarium conglutinans* on agar was the same. The optimum temperature for tomato wilt due to *Fusarium bulbigenum* var. *lycopersici* was found by Edgerton and Moreland (1920) to be 29° C. and by Clayton (1923) to be 29° C. and the optimum temperature for the growth of the fungus on agar was determined by Clayton as 28° C. The optimum temperature for the development of flax wilt was found by Jones and Tisdale (1922) to be 24–28° C. with an optimum of 26–28° C. for the growth of the causal fungus, *Fusarium lini* on agar.

Bewley (1922) found the optimum temperature for the development of tomato wilt due to *Verticillium albo-atrum* to be 21–23° C.; at 25° C. and above, development of the disease was inhibited. Walker and Jones (1921) found the optimum temperature for the development of onion smut to be 19–22° C. Richards (1921, 1923 a) determined the optimum temperature for the development of stem canker of potatoes due to *Rhizoctonia solani* as 18° C., above 24° C. little damage was caused by this parasite. The optimum temperature for growth of *R. solani* in pure culture is approximately 25° C., but stem canker damage was found to be worst at 18° C. not only in potato but also in cotton, pea and bean (Richards, 1923 b). Some strains of this fungus are known, however, to have a much higher optimum temperature for disease production; LeClerc (1934) found the

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\* This work was carried out at the Plant Pathological Laboratory, Sylhet.

optimum temperature for development of root rot in sugar beet to be 25–33° C. and Vasudeva and Ashraf (1939) demonstrated that the root rot of cotton in the Punjab was most severe at 35° C.

Tobacco root rot due to *Thielaviopsis basicola* was found by Johnson and Hartman (1919) to develop best at 17–23° C., at 26° C. the damage was considerably reduced and at 29° C. the amount of infection was negligible. Dickson (1923) in a classical study of the seedling blight in wheat and corn demonstrated that a single fungus, *Gibberella saubenetii*, might have widely different temperature optima for disease production in different crops, viz., wheat and corn. Whereas seedling blight developed to the greatest extent in corn at low soil temperatures (8–16° C.), the disease affected wheat seedlings most severely at relatively high soil temperatures (16–28° C.). The optimum temperature for the development of the disease caused by *Helminthosporium sativum* in wheat seedlings was found by McKinney (1923) to be about 28° C. From a review of all available experimental evidence Garrett (1942) has concluded that the take-all disease of wheat due to *Ophiobolus graminis* is favoured by high soil temperatures with an optimum around 25° C.

*Sclerotium rolfii* Sacc. is a soil dwelling fungus and is known to be parasitic on a number of our economic crop plants (Butler and Bisby, 1931; Uppal, *et al.*, 1935). In Assam it has been reported to be responsible for a very serious disease of pan (*Piper betle*, L.) in certain pan-growing tracts of Sylhet (Chowdhury, 1945). But hitherto no studies have been made anywhere to determine the optimum temperature for the development of diseases caused by this parasite. The following experiments were, therefore, carried out to determine the influence of temperature on the growth of *S. rolfii* and the effect of soil temperature on the development of sclerotial wilt of pan.

## II. EXPERIMENTAL

To obtain constant temperatures a number of Hearson incubators fitted with thermostatic capsules and designed to maintain definite constant temperatures with reasonable accuracy were used. Temperatures higher than the room temperature were obtained by warming those incubators designed to maintain such temperatures by electricity while temperatures lower than the room temperature were obtained by cooling those meant to give such temperatures with cold water and ice. Constant temperatures of 10° and 15° C. were obtained by the use of frigidariæ.

(i) *Effect of Temperature on the Growth of the Organism.*—The effect of temperature on the growth of the organism was determined by growing

the organism, in triplicate, in petri dishes on oat agar at constant temperatures. A single sclerotium was placed at the centre of each petri dish and the diameter of the colonies determined at the end of 5 days. The organism grew most rapidly at 28° C. No growth occurred at 10° and 40° C. during the 5-day period. A very small amount of growth, however, occurred at 10° C. after 12 days and at 40° C. after 7 days.

The average diameter of the colonies at the different temperatures is presented in Table I and in Fig. 1.

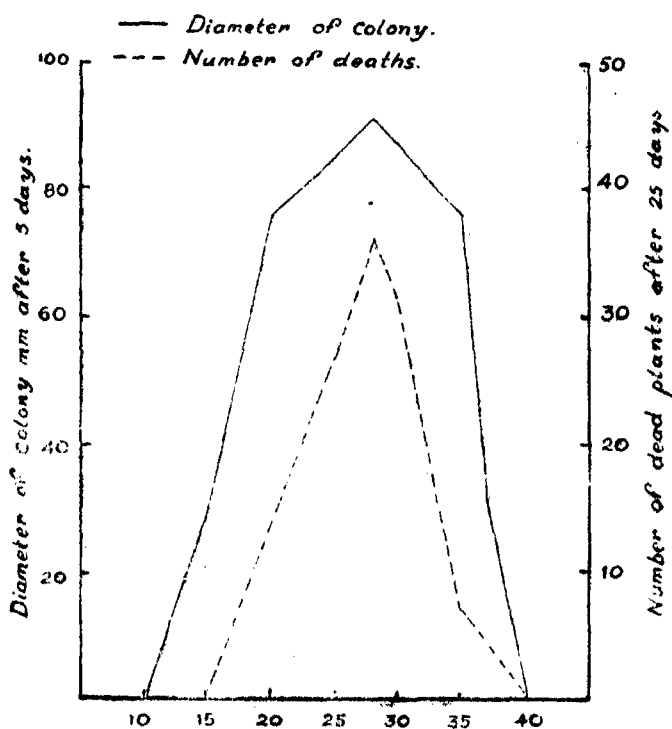


FIG. 1. Growth of *S. rolfsii* in culture and the percentage of death of *pan* plants at different temperature.

TABLE I

*Diameter of colonies in millimeter of S. rolfsii at various temperatures after 5 days*

Temperature	Diameter of colonies	Temperature	Diameter of colonies
10° C.	..	28° C.	91
15° C.	28	30° C.	87
20° C.	76	35° C.	76
25° C.	85	40° C.	..

(ii) *Effect of Temperature on the Development of Sclerotia of the Organism.*—To study the effect of temperature on the development of sclerotia the fungus was grown on oat meal and Dox agars and incubated at different temperatures for a period of 20 days. The intensity of sclerotia formation observed is recorded in Table II.

TABLE II  
*Formation of sclerotia of S. rolfsii at different temperatures*

Temperature	Sclerotia Formation		Temperature	Sclerotia formation	
	Oat agar	Dox agar		Oat agar	Dox agar
10° C.	..	..	28° C.	+++	+++
15° C.	+	++	30° C.	+++	+++
20° C.	++	++	35° C.	+	+
25° C.	++	++	40° C.	..	..

It will be evident from the data presented in Table II that the sclerotial formation took place over a wide range of temperature but the most suitable temperature for their development was 28° and 30° C.

(iii) *Effect of Temperature on the Germination of Sclerotia.*—The effect of temperature on the germination of sclerotia of *S. rolfsii* was studied by placing 20 days' old sclerotia on thin platings of Dox agar and examining them after regular intervals. The results obtained are recorded in Table III.

TABLE III  
*Effect of Temperature on the Germination of Sclerotia of S. rolfsii*

Temperature	Percentage of germination after hours					
	12 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
10° C.	..	..	..	..	..	..
15° C.	..	..	..	..	40	70
20° C.	..	..	..	90	..	..
25° C.	..	55	95	100	..	..
28° C.	..	70	100	..	..	..
30° C.	..	60	100	..	..	..
35° C.	..	..	100	..	..	..
40° C.	..	..	..	..	..	..

It will be evident from the data presented in Table III that the most suitable temperature for the germination of the sclerotia was 28° C., temperature 25° and 30° C. were only very slightly less favourable than 28° C.

(iv) *Effect of Temperature on Disease Development.*—For determining the effect of soil temperature on the development of sclerotial wilt of *pan*, three months' old *pan* plants growing in galvanised iron cans (10 plants per can) were inoculated with 20 days' old sclerotia of *S. rolfsii* and maintained at constant soil temperatures for 20 days. Constant soil temperatures were maintained by the use of Tanks of the Wisconsin Type (Jones, *et al.*, 1926). The inoculation technique was the same as described elsewhere (Chowdhury, 1945). At the end of 20 days the total number of deaths was noted. For each temperature 10 cans were used, that is 100 plants were subjected to the treatment. The number of deaths observed is recorded in Table IV.

TABLE IV

*Percentage of death of pan plants inoculated with S. rolfsii and grown at different soil temperatures for 20 days*

Total number of plants at each Temperature	Percentage of mortality after 20 days at soil temperatures of :						
	15° C.	20° C.	25° C.	28° C.	30° C.	35° C.	40° C.
100	..	14	27	38	31	4	..

It will be evident from the data presented in Table IV, that there were no deaths at soil temperatures of 15° and 40° C. The maximum number of deaths occurred at 28° C.; at 30° C. the percentage of death was less than that at 28° C. but was slightly more than that at 25° C. At 20° C. the number of deaths was more than that at 35° C.

Control plants kept remained all throughout healthy. At 40° C. though there were no deaths in the inoculated plants still the plants were not healthy and thrifty in their look. Plants maintained as controls at a temperature of 40° C. also presented the same sickly appearance. As such it might very probably be concluded that a soil temperature of 40° C. was too warm for the normal healthy growth of the *pan* plants.

The growth of the fungus in culture and the number of deaths of the *pan* plants as it occurred at the different temperatures due to the attack of *S. rolfsii* are presented graphically in Fig. 1. It will be manifest from this figure that the temperature for the maximum growth of the organism in culture agrees with that for the maximum disease development as both of these occurred at 28° C.

### III. SUMMARY

The effect of temperature on the growth of *S. rolfsii* and the effect of soil temperature on the development of sclerotial wilt of *pan* were studied.

It was found that the optimum temperature for the vegetative growth of the organism in culture was 28° C. and the maximum number of sclerotia was formed at 28° and 30° C. in culture. The optimum temperature for the germination of sclerotia in Dox agar was observed to be 28° C.

Optimum soil temperature for the development of the disease was found to be 28° C., although at 25° and 30° C. the disease was quite severe.

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